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Influences of Different Processing Technologies on the Properties of Emulsion-Type Sausages.

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INFLUENCES OF DIFFERENT PROCESSING TECHNOLOGIES ON THE PROPERTIES OF EMULSION-TYPE SAUSAGES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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ABSTRACT

Emulsion-type sausages were manufactured for evaluation of microstructure, smokehouse yield, and textural characteristics with different lean sources, salt levels, casing sizes and endpoint cooking temperatures. Emulsion stability and water binding capacity of raw batters manufactured with 1, 2 or 3% salt were examined at chopping temperatures of 10, 15.5 and 21°C. Frankfurters, manufactured with three sources of pork fat, were examined during a nine week storage for oxidative rancidity under different packaging conditions. Bologna from forage-finished beef had lower water, fat and total losses and a higher smokehouse yield than bologna from grain-fed beef at 1 and 2% salt levels. Emulsions with 1 or 2% salt had lower emulsion stability and water binding capacity, lower smokehouse yield and a softer texture than emulsions with 3% salt. Less denaturation occurred in 13.6 cm casings than in 9.6 cm casings. The endpoint cooking temperatures of 74°C reduced smokehouse yield in low salt treatments compared to 67°C temperatures. SEM micrographs indicated that bologna with higher salt contents had a thicker protein coating around the fat globule and a more stable emulsion compared to lower salt treatments. A desiccator drying technique after chemical fixation of bologna samples for SEM was

judged to be equal to the commonly used critical point drying technique. Cryofracturing without fixation was not satisfactory in allowing specific identification of fat and protein components in bologna specimens. Greater oxidative stability of frankfurters was obtained with vacuum packaging during extended storage times. Frankfurters manufactured with prerigor (warm) fat had lower TBA numbers compared to postrigor (chilled) fat and lard treatments.

INTRODUCTION

Sausage, which is derived from the Latin term salsus, meaning salt, is a ground meat product that is salted and seasoned. Sausage is one of the most popular meat products in the U. S. today. In 1984, 1,405,258,000 pounds of frankfurters and 688,220,000 pounds of bologna were produced under federal inspection (Knutson, 1985). Sausages can be separated into ground sausages and emulsion-type sausages. Ground sausages show discrete particles of lean and fat while in emulsion-type sausages, fat is emulsified and stabilized by the lean meat in a homogeneous mixture. Many processing variables, including the amount of lean meat used, type and level of fat added, mechanical treatment, thermal processing and salt content, influence the texture, flavor, yield, and stability of emulsion-type sausage products.

The level of sodium chloride in processed meat products has been a great concern because sodium intake seems closely related to human hypertension. Salt is used in emulsion-type sausages to increase water binding capacity, emulsion capacity, emulsion stability and other functional properties. These sausage characteristics have a close relationship to structural integrity. Scanning electron microscopy (SEM) is a technique which allows the

microstructure of food to be examined in great detail. However, there has been limited usage of SEM in the quality evaluation of processed meat products because the identification of easily recognizable biological microstructures in the meat emulsion is difficult. Little reference information is available on the microstructure of low-salt emulsion-type sausage products. Also, the influences of other processing variables, such as casing sizes and endpoint cooking temperatures, on smokehouse yield and textural characteristics are not clear. Prerigor meats have greater emulsifying and water holding capacity and usually lower microbial counts than postrigor meats. However, most prerigor processing research has measured the effects of muscle rather than fat contributions to meat properties.

The purpose of this dissertation research was to investigate the influences of different processing technologies on the properties and microstructures of emulsion-type sausage products. The body of the dissertation is divided into four experiments, corresponding to four areas of research interest, and written in journal research format suitable for publication.

In experiment one the influences of salt level, casing size and endpoint cooking temperature on SEM microstructure, yield and texture characteristic of bologna were studied. Experiment two resulted from the knowledge gained in the first experiment and was performed to study the effect of

salt content on bologna properties and microstructure in more detail.

Artifacts in the bologna microstructure may be introduced during sample preparation. The purpose of experiment three was to compare the microstructure of cryofractured bologna specimens prepared with different fixation techniques. The effects of different fat sources, packaging conditions and storage periods on the oxidative stability in frankfurters were evaluated in experiment four.

LITERATURE REVIEW

STRUCTURE OF MEAT

There are three basic types of protein in muscle: myofibrillar, sarcoplasmic and stromal. The myofibrillar proteins constitute about 50 to 55% of the total protein content. The myofibrillar proteins are salt-soluble and are mainly responsible for the functional properties in a comminuted meat emulsion (Schut, 1976; Acton et al., 1983). The sarcoplasmic proteins account for about 30 to 34 % of the total protein content. The sarcoplasmic proteins are water-soluble and serve as enzymes and heme pigments in the muscle cells (Forrest et al., 1975). The remaining 10 to 15% of total proteins are the stromal proteins or the connective tissue (collagen). The stromal proteins are soluble in high salt concentration and have unique characteristics. Collagen shrinks and converts to gelatin upon thermal processing (Forrest et al., 1975).

Animal fats are composed mainly of neutral lipids (triglycerides) and phoshoplipids. A triglyceride is an ester of three fatty acids and glycerol. Triglycerides are generally considered as the major portion of meat fat. Phospholipids, which include compounds such as lecithin, cephalin, and sphingomyelin, are found in animal fats in

small amounts. They play a major role as structural and functional components of cells and membranes (Kramlich et al., 1975; Ockerman, 1980). Forage-finished beef contains less unsaturated fatty acids and more polyunsaturated fatty acids as compared to grain finished beef (Williams et al., 1983). Williams et al. (1983) indicated that the lower amounts of stearic acid and higher amounts of oleic acid accounted for more unsaturation of fatty acids in beef from cattle finished on grain than those finished on forage. Steers finished on forage yielded meat with higher amounts of polyunsaturated linoleic and linolenic acids than steers finished on grain.

MEAT EMULSION DEFINITIONS

A meat emulsion is a two-phase system, which consists of a fairly coarse dispersion of two immiscible liquids, one dispersed in the other. The two phases of an emulsion are referred to as continuous and discrete (Forrest et al., 1975; Kramlich et al., 1973). Most emulsions involve water and oil or fat as the two immiscible phases. Sausage products, in some respects, resemble true oil-in-water emulsions, which contain a fat-discrete and a water-continuous phase. The emulsifying agents are salt-soluble proteins, which stabilize the fat dispersion and form the emulsion matrix (Saffle, 1968). Sufficient chopping is necessary to create the dispersion of fat droplets in the

meat emulsion (Saffle, 1968). Hansen (1960) indicated that the salt-soluble proteins are coagulated around the fat droplets upon thermal processing, creating a stable emulsion. Microscopic examination of heat-processed sausages has shown that under optimal conditions, most of the fat cells in an emulsion maintain their integrity despite extensive grinding or chopping. The major part of the fat in a sausage emulsion was found to be dispersed as intact cells or cell clusters (Evans et al., 1975). Schut (1976) indicated that meat emulsions involve a multiphasic system where fat is dispersed in a complex matrix of soluble proteins, segments of muscle fibers, connective tissue and salts. It has been proposed by Brown (1975) and Terrell et al. (1980) that the term "meat batter" be used instead of "meat emulsion" to describe finely comminuted raw sausages.

MEASUREMENTS OF EMULSION CHARACTERISTICS

EMULSION CAPACITY AND EMULSION STABILITY

The emulsion capacity can be defined as the maximum amount of fat or oil with which a given amount of meat will combine to form an emulsion; however, the ability to bind fat does not necessarily mean that the fat cells remain stable during heat processing. In a more practical sense, emulsion stability is more suitable to describe the stability of meat emulsions. The emulsion stability refers to the ability of the meat emulsion to bind fat and water

until completion of processing. Emulsion stability is influenced by many factors including the water binding capacity of the proteins, fat holding capacity of the protein matrix, the level of meat, water, fat, and nonmeat additives in the formulation, mechanical treatment and heat treatment (Schut, 1976).

Trautman (1964) failed to emulsify fat with water-soluble proteins, but Carpenter and Saffle (1965) indicated that water-soluble proteins had a greater emulsifying capacity than had been previously reported. Water-soluble proteins have a spherical, rigid shape, and have an emulsifying capacity of 29-30 ml of oil per 100 mg of soluble protein as compared with 39-42 ml of oil per 100 mg of salt-soluble proteins (Carpenter and Saffle, 1965).

WATER BINDING CAPACITY

Water binding capacity in muscle tissue is the ability of meat protein to bind its own and added water (Ockerman, 1980). Price et al. (1975) reported that muscle proteins are the principal water binding substances in meat.

Three types of water, known as bound water, restricted water, and free water, exist in skeletal muscle. According to Hamm's (1960) estimation, 70% of the water content in fresh meat is located within the myofibrils, 20% in the sarcoplasm, and 10 % in the connective tissue. Only a small amount of total water in muscle is tightly bound to the

muscle protein so the major influence of protein charges and protein structure is on the free water in meat. Most of the free water is immobilized in a network of membranes and within the filamentous proteins. Free water that is not immobilized constitutes the loose water, which is expressed when water binding capacity decreases (Price et al., 1975; Ockerman, 1980.). The water binding capacity is mainly influenced by pH, salt addition, onset of rigor mortis, aging, and species (Lawrie, 1974; Acton et al., 1982). The water binding capacity is lowest when pH reaches the isoelectric point (pH 5.0-5.2), but on either side of the isoelectric point, the water binding capacity is increased (Forrest et al., 1975).

The addition of sodium chloride usually increases the water binding capacity. The chloride ions of salt can form a complex with muscle protein and at the isoelectric point, sodium chloride increases the water binding capacity and swelling of meat fibrils (Price et al., 1975). Prerigor meat has a higher water binding capacity as compared to postrigor meat due to its higher pH. At higher pH, the amount of protein extracted is increased. The amount of salt-soluble protein was 50% greater in prerigor beef than in beef 48 hour post-mortem. Freezing beef reduced the salt-soluble protein in comparison with beef 48 hour post-mortem (Saffle and Galbreath, 1964). Meat of young animals usually has a higher water binding capacity than meat from older animals (Schut, 1976). Pork usually has a higher

water binding capacity than beef; however, the comparison must be made in muscles from similar locations of the beef or pork carcasses (Hamm, 1960).

EFFECTS OF PROCESSING VARIABLES ON THE EMULSION STABILITY AND WATER BINDING CAPACITY OF MEAT EMULSIONS

COMMINUTION OF MEAT EMULSIONS

Grinding, mixing, and chopping (emulsifying) are three major steps in making a meat emulsion. Grinders are utilized to reduce the size of meat into uniform cylinders of fat and lean. Cylinders of fat and lean obtained by grinding are tumbled in a mixer to give a uniform distribution of fat and lean particles. A silent cutter or a high-speed emulsifier is often used to chop and form the meat emulsion. Although the major differences in comminuted meat products are due to mechanical treatment during comminution (Schut, 1976; Schmidt et al., 1981), research describing the effects of different machines on the functionality of the meat ingredients is limited.

Chopping causes simultaneous size reduction, salt soluble protein extraction, and emulsification (Kramlich et al., 1975). The temperature of the meat batter during chopping will rise 5.6° to 11.1°C in 10 to 15 min of chopping. With continued chopping the fat particle size is

decreased and the number of fat particles is increased. Mechanical entrapment of these fat particles in a stable matrix of protein, fat and water is probably responsible for the stability of a comminuted meat batter.

The average distance between fat droplets has been referred to as the interfacial film thickness. The stability of emulsions is dependent on the relationship of the interfacial film thickness to droplet size as well as the droplet's size in the disperse phase. Ivey et al. (1970) suggested that when the droplets decreased in size, greater interfacial film thickness was needed to decrease coalescence. If the droplets were too closely packed, they coalesced and the oil was released. For maximum stability, lean meats have commonly been comminuted to a minimum temperature of 3^{o}C and maximum of 18^{o}C. Comminution of lean tissue must be sufficient to disrupt membranes and sarcolemma for freeing of myofibrils and to bring the myofibrillar fraction to a high degree of swelling (Acton et al., 1982). Optimum fat and water binding in chopped batters appear to be very strongly dependent upon the final temperature of chopping (Brown et al., 1975). There is a close relationship between water binding capacity and fat binding capacity during the comminution of a sausage preparation (Brown et al., 1975). The water binding capacity and fat binding capacity both decrease in the initial comminution phase and then increase after continuous chopping. As chopping temperature reached 20^{o}C, both water

binding capacity and fat binding capacity decreased. Hansen (1960) reported that chopping emulsions to a final temperature of 11°C was not high enough to disperse all of the fat particles, while a final temperature of 27°C resulted in a broken protein matrix and fat separation. Swift et al. (1961) reported that the amount of fat, which was emulsified decreased with an increase in final chopping temperature and found that maximum emulsification was obtained at 18°C. Helmer and Saffle (1963) found that emulsions were stable at chopping temperatures of 15.5°C or lower, but higher temperatures caused unstable emulsions. Webb et al. (1975) studied the effect of lipid and chopping temperatures on sausage emulsion stability. They reported that a low extraction temperature (5°C or lower), high temperature for fat addition (76.6°C), and chopping temperature lower than 25°C was required to form a stable emulsion. It had been suggested by Webb (1974) that blending and chopping temperatures are extremely important. The lean tissue must be blended with water and salt at a low temperature near the freezing point and meat particle size must be reduced sufficiently to allow protein extraction. Brown and Toledo (1975) concluded that the temperature range of maximum binding in meat batters was 15 to 22°C and that prolonged chopping resulted in changes in the fat and water binding regardless of temperature control. This supported the statements of Price et al. (1971) that prolonged chopping of both lean and fat tended to decrease, rather

than increase, emulsion stability. Townsend et al. (1968), using differential thermal analysis, determined that there were two primary melting ranges of fat. The ranges were from 3° to 14 °C and from 18° to 30°C for beef fats, and 8° to 14°C and 18° to 30°C for pork fats. They concluded that emulsions chopped to more than 18.5°C were unstable. Townsend et al. (1971) suggested that emulsions containing beef fat should be comminuted to 18° to 24°C to avoid possible under- or overchopping. Ackerman et al. (1967) reported that pork fat had greater emulsion stability because it was more widely dispersed than beef fat. Morrison et al. (1971) proposed that emulsion instability was highly dependent upon the level of added water. At a 30% fat level, there was a sharp drop in stability as added water was reduced below 16% for fresh beef, whereas an equivalent drop in stability was found as added water was reduced below 21 % in frozen beef.

THERMAL PROCESSING

Cooking meat and meat products serves to coagulate and denature the meat proteins, improve meat palatability, inactivate enzymes in meat and prevent off-flavor, improve the shelf-life of meat products, decrease the water content of meat products, stabilize the red color in cured meat and modify the texture (Kramlich et al., 1975). Denaturation and coagulation involve changes in the protein

molecule. The molecular changes in meat proteins, such as rupturing of hydrogen bonds, unfolding of peptide chains, and altering of random coil configuration, are generally considered as denaturation. An extensive unfolding of side chains, as occurs during heating, causes further aggregation and precipitation of meat proteins. This process is called coagulation which is an extensive denaturation. Heating of meat also causes shrinkage. This is due to the thermal shrinkage of both muscle fibers and collagen fibers (Laakkonen, 1973). The volume of the muscle fibers has been shown to decrease in the temperature ranges from 45°C to 80°C with the most rapid rate of decrease between 50° and 65°C. Collagen shrinkage occurred at temperatures of 58 to 62°C (Hostetler and Landmann, 1968). Collagen shrinkage is caused by a rupture of the interchain crosslinkages of collagen and generally occurs in the temperature range of 58° to 62°C (Gustavson, 1956).

Upon heating, meat protein decreases its solubility and increases its rigidity. Myosin plays a major role in completely surrounding the fat particles. Hamm (1966) suggested that at temperatures between 35° and 50°C, the actomyosin molecules become unfolded and coagulation begins at 35°C. At temperatures above 70°C, unfolding is completed. The decreased solubility of the sarcoplasmic proteins during heating indicates that the sarcoplasmic proteins coagulate at temperatures between 40° and 60°C (Hamm, 1966).

The gelling of muscle proteins during heat processing is mainly responsible for the texture development in comminuted products (Acton et al., 1982). The suspension of macroparticles in the raw emulsion batter are transformed into a gel matrix by temperature changes during heat processing. Further heating of the batter during thermal processing coagulates the protein and stabilizes the emulsion. The coagulated proteins hold the fat in suspension and form a stable texture (Kramlich et al., 1975).

Myofibrillar proteins have excellent gel-forming abilities. Siegel and Schmidt (1979) observed that there were no differences in the binding abilities or gel structure between myosin and actomysin. Montejano et al. (1984) monitored the gelling process during heating of beef, pork, and turkey. They indicated that beef had major rigidity increases at 43°, 56°, and 69°C; pork at 44°, 53°, and 69°C; and turkey at 50°, 53°, and 79°C. Kempton and Trupp (1983) reported that in raw emulsions, most of the fat globules appeared to be closely surrounded by a coating of protein, but after cooking the fat globules were described as irregularly shaped particles and most of the fat remained trapped within a protein matrix. Emulsion stability and water binding capacity decrease with increasing temperature; however, oil does not generally separate from the emulsion during thermal processing, which indicates that oil droplets are stabilized in the protein

matrix structure (Schut, 1976). Swasdee et al. (1983) indicated that collagen swelled and was completely denaturated at 70°C. This may be a factor contributing to loss of binding of fat and water of cooked meat batters. Sausages with high collagen content from pork stomachs or beef tripe have an imbalance of myosin to collagen in the meat components. Fat particles covered with collagen-type protein are stable after emulsification. However, upon heating, collagen shrinks, converts to gelatin and results in an unsatisfactory product with a fat cap at the top and a jelly pocket (gelatin solution) at the bottom of the sausage. Kramlich (1965) reported that in formulations utilizing skins and tripe, which contain high amounts of connective tissue, emulsion instability was observed and associated with gelatin caps or pockets. Fat separation may occur as a result of heating too rapidly or because of high temperatures. The coagulated protein coating ruptures as the fat particles expand on continued heating and results in a small amount of fat at the surface of emulsion-type sausages. Trout and Schmidt (1986) indicated that water binding capacity was maximum at 56°C and decreased as the cooking temperature increased, but differing fat levels had no effect on the water binding capacity with the same effective salt concentrations. They also pointed out that salt in meat products resulted in a higher coagulating temperature of meat proteins; therefore, more water could be immobilized in the protein matrix before the meat proteins

coagulated.

High temperature and high relative humidity have adverse effects on emulsion stability, texture and color development (Saffle et al., 1967; Kramlich et al., 1975; Monagle et al., 1974). Monagle et al. (1974) reported that the rate of heating of sausages was faster at 80% relative humidity compared to 20% relative humidity and for the same relative humidity, heating was faster at slower air flow rates. A low relative humidity process showed more moisture loss and a slower heating rate than a high relative humidity process (Monagle et al., 1974), and frankfurters cooked slightly faster in a high humidity atmosphere. Keeton et al. (1984) indicated that frankfurters processed to higher temperatures (82^{o}C) had lower smokehouse yields than frankfurters processed to lower temperatures. Prusa et al. (1985) indicated that a rapid heating rate of emulsion by electrical resistance at 40 volts and the addition of sodium chloride increased batter expansion and raised the temperature at which the batters reached maximum expansion compared to a slow heating rate. Furthermore, batter contraction was decreased with increased heating rate and the addition of sodium chloride. In the expansion of batters, more water appears to be bound which increased the emulsion stability. This agreed with Acton et al. (1983) that protein aggregation is dependent on both a temperature increase and an increase in the rate of heating. Voutsinas et al. (1983) reported that heat denaturation of

some food proteins such as soy isolate did not always cause a loss of emulsifying capacity, but could result in great improvement.

INFLUENCE OF SALT ON EMULSIONS

Salt (sodium chloride) serves three functions in sausage: preservation - it dissolves in water and forms a brine, which retards microbial growth; flavor - it contributes to basic taste characteristics; and protein solubilization - it aids in solubilizing the myofibrillar proteins of comminuted muscle for fat emulsification in emulsion-type sausages (Forrest et al., 1975; Price et al., 1970; Kramlich et al., 1975). In emulsion-type sausage products, salt influences product flavor and extracts and solubilizes muscle proteins which emulsify and bind fat, moisture and other ingredients (Rust, 1976; Saffle, 1968). The salt-soluble proteins form a matrix which coagulates during heat processing to result in acceptable product yield, texture, bite, moistness, appearance, overall quality and product identity (Schmidt et al., 1981). The negatively-charged chloride ions screen the positively-charged protein groups of meat and result in repulsion of the protein and an enlargement of the space for more water absorption (Schut, 1976).

The level of sodium in processed meat products has been a health concern since processed meat products are

considered a major source of sodium in the human diet (Sofos, 1983; Hand et al., 1982). Processed red meat products usually contain 2.25 to 2.75% NaCl in their formulation (Olson and Terrell, 1981) and so sausage products frequently contain more than 300 mg of sodium per serving. Muscle foods (red meat, fish, and poultry) will contribute 15 to 25% of the sodium to an average diet (Andres, 1982). The sodium ion seems closely related to human hypertension (Sebranek et al., 1983) so the reduction of salt content in processed meat products has been an important meat research interest. Offer and Trinick (1983) reported that reduction in the NaCl content of emulsion-type sausages influenced water binding capacity, emulsion capacity, emulsion stability and other functional properties. Several approaches have been used to study the effects of reducing the salt content, including lowered levels of NaCl, replacing part of the NaCl with other chloride salts or nonchloride salt compounds, and altering processing techniques (Terrell, 1983).

Sofos (1983a) examined the reduction of NaCl without substitution in frankfurters manufactured under commercial conditions and concluded that a salt concentration of 2 to 2.5% was necessary for the manufacture of commercial frankfurters without any other substitution. Reduction of NaCl level from 2.5% to either 1% or 1.5% resulted in reduced emulsion stability and increased smokehouse losses. Microbial growth was only slightly more rapid in

frankfurters made with lower salt levels than with higher salt levels. Sebranek et al. (1983) stated that a reduction in NaCl up to 40% would not appear to increase potential pathogenic growth. Sofos (1983b) concluded that a reduction of salt levels in frankfurters by 20% or more resulted in products with lower smokehouse yield and emulsion stability, undesirable flavor, texture and overall acceptability and reduced shelf-life compared to products containing normal salt levels.

Whiting (1984a), in his study of stability and gel-strength of frankfurter batters made with reduced NaCl, indicated that 1.5 % salt batters had greater water losses and lower emulsion stability than 2.5% salt batters. Water binding capacity was enhanced by preblending prerigor meat. Puolanne and Terrell (1983a and 1983b) had previously reported that reducing salt level from 2.5 to 1.5% did not affect physical, chemical or sensory properties of emulsion-type sausage made with preblends of prerigor pork with 2% salt. They suggested that preblending of prerigor meats was beneficial for optimizing water binding capacity of sausage made with low salt content.

Partial replacement of NaCl with KCl and other chloride salts in processed meats has had some success (Seman et al., 1980; Terrell et al., 1981); however, flavor differences, mainly bitterness, have been a limiting factor. Use of KCl to replace up to 35% of the NaCl resulted in no differences from controls (100% NaCl) in flavor, texture, and moistness

while the use of $MgCl_2$ resulted in slight off-flavors as compared to controls (Hand et al., 1982).

There are certain techniques that will allow reduction of salt such as the use of phosphates, pre-blends and pre-emulsified fats (Rust and Olson, 1982). The use of phosphates as a partial replacement of NaCl in meat products has been suggested (Pepper and Schmidt, 1975; Trout and Schmidt, 1984). Hamm (1970) discussed the effect of phosphates on the increase in water binding capacity and suggested the possible modes of action as raising the pH, increasing the ionic strength, dissociating actomyosin and binding to meat protein. Since the pyrophosphate was involved in the dissociation of actomyosin, use of pyrophosphates resulted in a reduction of emulsion viscosity and a reduction of frictional heat buildup (Toth and Hamm, 1969). Subsequently, the rate of emulsion temperature increase during chopping was less with the addition of phosphates than with no phosphates. Hargett et al. (1980) reported that sodium acid pyrophosphate was the most effective phosphate for improving the firmness of frankfurters. Knipe et al. (1985a) indicated that the addition of inorganic phosphates enhanced water binding capacity and emulsion stability. Sodium pyrophosphate and potassium pyrophosphate resulted in more stable emulsions than sodium tripolyphosphate and potassium tripolyphosphate. Another study by Knipe et al. (1985b) suggested that the addition of NaOH and phosphates increased emulsion pH and

reduced emulsion viscosity; therefore, water binding capacity, emulsion capacity, and protein solubility were all enhanced.

Use of pre-emulsified fat in meat emulsions has been reported by Zayas (1985) and Lin and Zayas (1987). Use of 3% sodium caseinate or 5% nonfat dry milk to pre-emulsify fat resulted in more uniform dispersion of fat droplets in the emulsion structure, which increased water binding capacity and smokehouse yield of frankfurters (Lin and Zayas, 1987). Corn germ protein stabilized meat emulsions and prevented fat coalescence during thermal processing. Enzymatic modification has also been used to improve the functional properties of low-grade proteins for specific processing needs (Richardson, 1977). Use of enzymatically modified mechanically deboned fowl resulted in improved texture, color, and flavor as compared to commercial chicken frankfurters (Smith and Brekke, 1985).

DIFFERENCES IN FAT SOURCES

EFFECT ON MEAT EMULSIONS

The effects of fat on the properties of sausage emulsions have been studied by several researchers. Ackerman et al. (1971) reported that dispersion of cottonseed oil produced finely dispersed particles with a substantial number of particles less than 1 um in diameter.

Fat tended to separate from frankfurters containing beef fat where particles were 200 um or more in diameter. Interchanging the type of emulsified oil among corn, soybean, safflower and peanut oil did not alter emulsion stability (Acton and Saffle, 1971).

Tadic (1966) compared emulsions of animal fat tissues and rendered fats derived from the same location of the animal and reported that there was greater stability of emulsions produced with fat tissues, whereas the emulsions with rendered fat showed a finer fat particle size. Christian and Saffle (1967) found that a greater amount of short chain fatty acids were emulsified than longer chain fatty acids, fatty acids with one double bond were emulsified more than fatty acids with two double bonds and saturated fatty acids were less emulsified than unsaturated fatty acids. Their results indicated that when the length of the carbon chain is the same, unsaturated fatty acids (with one or two double bonds) were less emulsified than saturated fatty acids. Schut (1976) concluded that at higher fat contents, the high melting fats were shown to produce stable emulsions, and the low-melting oily fractions produced unstable emulsions. Egelandsdal et al. (1985) reported that increasing aliphatic chain length of fatty acid salts decreased thermal stability of myosin extracts, but enhanced gel strength. They suggested that increased repulsion between protein chains led to a more ordered aggregation (gelation) during thermal processing.

LIPID OXIDATION

Animal fats are composed mainly of neutral fats and phospholipids. The neutral fats are principally triglycerides. Phospholipids are found in animal fats in small percentages but play a key role as structural and functional components of cells and membranes. Phospholipids are more readily oxidized than triglycerides; therefore, they play an important part in development of off-flavors and undesirable odors in meat products (Kramlich et al., 1975).

Lipid oxidation has been a major cause of deterioration in the quality of stored meat and meat products (Love, 1983). One of the major concerns with animal fats is their susceptibility to oxidize and influence subsequent meat color, flavor, and functionality of muscle proteins. The oxidative deterioration of muscle lipids involves oxidation of the unsaturated fatty acids. Oxidation is the reaction of oxygen with the double bonds of the fatty acids. The more unsaturated the fat the more susceptible it is to oxidation (Ockerman, 1980). The polyunsaturated fatty acids with three or more double bonds (phospholipids) are associated with the development of off-flavor in muscle (Allen and Foegeding, 1981). The oxidative reaction produces intermediates known as hydroperoxides or primary products of oxidation. The peroxides then break down to carbonyls or react with other compounds such as proteins,

vitamins, or other natural constituents (Melton, 1983). The end products of the oxidative reaction are short chain aldehydes and short chain acids which have a very offensive odor and flavor (Ockerman, 1980; Price et al., 1975). The following factors affect the rate of oxidation in muscle lipids: oxidation occurs more rapidly with higher degrees of unsaturation; hydrogenation reduces unsaturation and consequently oxidation; ultraviolet light increases oxidation and strong display lights increase the speed of oxidation; an increase in pH in the muscle causes a reduction in oxidation; removal of oxygen or vacuum packaging retards oxidation; and the addition of antioxidants in small amounts can decrease oxidation (Ockerman, 1980).

Younathan and Watts (1960) showed that the lipids involved in flavor deterioration in cooked meat were the unsaturated fatty acids of the lean tissue, primarily phospholipids. Wilson et al. (1976) found that phospholipids were major contributors to rancidity in beef; however, total lipids were more important than phospholipids in pork. Hornstein et al. (1961) concluded that phospholipids did not contribute to desirable meat flavor; however, they could contribute to undesirable flavor. In addition to affecting palatability factors, such as meat flavor and color, lipid oxidation produces compounds which have adverse biological effects.

The ability of heme compounds to catalyze lipid

oxidation is well known. Myoglobin is the major catalyst of lipid oxidation. Other heme compounds in red meats might function as prooxidants in muscle tissue (Love, 1983). It has been shown that both hemoprotein and nonheme iron components of meat are catalysts of lipid oxidation in model meat systems (Rhee, 1978). Love and Pearson (1974) demonstrated that in cooked meats, nonheme iron was a prooxidant of muscle lipids. Tichivangana and Morrissey (1985) demonstrated that free iron was released when myoglobin is denatured by heating, but that heme iron was not a major pro-oxidant in unheated muscle. Their results also confirmed the work of Wilson et al. (1976) that susceptibility to oxidation occurs in the order fish > turkey > chicken > pork > beef > lamb. Verma et al. (1985) reported that denatured myoglobin and denatured metmyoglobin were catalysts of lipid oxidation in meat emulsions, but that ferrous and ferric salts and 1.5% sodium chloride were weak catalysts of oxidation compared to ferric heme pigment complexes.

It has been assumed that lipid oxidation in muscle foods is nonenzymatic; however, there is evidence that there are lipid-peroxidation systems associated with muscle microsomes (Love, 1983). Lin and Hultin (1977) reported that a microsomal fraction prepared from chicken leg muscle was an enzymatic system for the oxidation of myoglobin to metmyoglobin.

ADDITION OF NITRITE

It is generally believed that nitrite functions to inhibit lipid oxidation and off-flavor in cured meat products. Zipser et al. (1964) proposed that nitrite reacts with heme-containing proteins to form catalytically inactive species. Nitrite also reacts with unsaturated fatty acids in animal fats. Sato and Hegarty (1971) suggested that nitrite might inhibit natural prooxidants present in muscle or stabilize the lipid components of the membranes (phospholipids). MacDonald et al. (1980) studied the effect of sodium nitrite on lipid peroxidation in unsaturated fatty acid emulsions and concluded that low nitrite concentrations (10 mg/kg) inhibited lipid oxidation, while a slight prooxidant effect was found at nitrite concentrations of 80 mg/kg or higher.

EFFECT OF HEATING ON LIPIDS

Lipids may undergo chemical changes such as hydrolysis and oxidation during heating. The effects of cooking are greater in pork and chicken than for beef. This has been attributed to differences in the quantity of polyunsaturated fatty acids (Allen and Foegeding, 1983). Siu and Draper (1978), in their survey of malonaldehyde content of retail meat products prior to and after cooking, reported that lipid oxidation was minimal in cuts that were cooked in a

short time. Large retail cuts had malonaldehyde levels of 2-10 times higher after cooking while steaks and chops cooked for shorter periods of time had no increase or a slight increase in malonaldehyde.

THIOBARBITURIC ACID (TBA) TEST

The most widely used test for measuring the extent of oxidative deterioration of lipids in muscle foods is the thiobarbituric acid test (TBA). The TBA test expresses lipid oxidation in mg of malonaldehyde per kg of sample, or TBA number. Malonaldehyde is one of the secondary carbonyl products of oxidized polyunsaturated fatty acids containing three or more double bonds. The TBA test may be performed directly on the food product, followed by extraction of the colored complex; on an extract of food; or on a portion of the steam distillate of the food (Melton, 1983). Rhee (1978) stated that the most popular method for measuring the TBA number in muscle foods is the steam distillate method of Tarladgis et al. (1960). However, several researchers have indicated that TBA numbers were higher when determined by the distillation method than when determined by the method using food extracts (Witte et al., 1976). Siu and Draper (1978) reported that TBA numbers with the distillate method were 1.4 to 1.7 times higher than with the extraction method. Recently, the utilization of high-performance liquid chromatography was proposed to determine specific

malonaldehyde compounds (Kakuda et al., 1981; Won Park and Addis, 1985).

SCANNING ELECTRON MICROSCOPY (SEM) OF MEAT EMULSION MICROSTRUCTURE

Visual observation has always played a major role in scientific methods. Since the commercial SEM became available in 1965, its use in meat science research has been applied to study differences in muscle tissue structure caused by aging, enzyme action and heating (Schaller and Powrie, 1971; Eino and Stanley, 1973; Leander et al., 1980).

There has been limited usage of SEM in the quality evaluation of processed meat products. The major drawback of using SEM has been in distinguishing fat from protein components (Ray et al., 1979) and the absence of easily recognizable biological structures (Ray et al., 1981). The globular structure of the lipid was often used as one major characteristic to distinguish it from protein (Basgall et al., 1983). This was confirmed by using fat stains and light microscopy (Ray et al., 1979).

Sample preparation for SEM requires fixation, dehydration and critical point drying. Simple chemical fixation was used by Jones and Mandigo (1982) in their study of meat emulsions. Cryofracturing followed by chemical fixation was used by Theno and Schmidt (1978) in their study of microstructure of three commercial frankfurters.

Critical point drying, which is rather time consuming, was employed in both studies. A desiccator drying technique which is useful for preparation of large numbers of samples was suggested by Basgall et al. (1983) instead of critical point drying.

The histological views of the protein matrix surrounding the fat globule in model emulsions and in commercial frankfurters were first shown by Hansen (1960). Sufficient chopping was required to form a protein matrix which encapsulated the fat globules and the salt-soluble proteins appeared to surround the fat globules to form a stabilizing membrane. The importance of salt soluble proteins to binding in emulsion-type products was demonstrated by Hansen (1960). Higher temperatures, associated with excess chopping, caused denaturation of the protein matrix and permitted unprotected fat globules to separate during cooking. The highly comminuted meat emulsion appeared to be structurally similar to an oil-in-water emulsion (Hansen, 1960; Swift et al., 1961; Helmer and Saffle, 1963). Some researchers showed that a dense membranous layer surrounded each fat globule. Borchert et al. (1967) examined meat emulsions using a transmission electron microscope (TEM) and found that the membrane surrounding the fat globules had some small pores or openings and the continuous phase of the emulsion had been altered by thermal processing. Fat droplets as small as 0.1 um with a distinct protein membrane were reported by

Borchert et al. (1967). Ray et al. (1981) observed the microstructure of liver sausage with SEM and reported that protein structures surrounded the fat globules of emulsified fat in a lace-like network. The fat, protein and air pockets in meat emulsions could be identified by using SEM (Ray et al., 1979).

Theno and Schmidt (1978) examined three commercial frankfurters and postulated that a true emulsion did exist in meat emulsions. They showed that the protein matrix and fat droplet size in commercially-produced frankfurters varied widely. Structural matrices in the frankfurters ranged from coarse protein fragments surrounding large fat particles to a uniform dispersion of small fat droplets in a fine protein network. In frankfurters, not all fat droplets are of uniform size and not all are uniformly surrounded by protein membranes (Borchert et al., 1967; Theno and Schmidt, 1978).

Lee et al. (1981) reported that in a gel-type emulsion the fat droplets are physically confined within the protein matrix and thus the shape of all fat droplets did not remain globular. A wide variation among the microstructures of meat emulsion was known to exist. The term "meat batter" rather than "meat emulsion" was suggested by Terrell et al. (1980) since emulsion-type sausages are multiphase systems in which salt, protein and water function both chemically and mechanically to stabilize the raw and cooked emulsion.

CHANGES IN MICROSTRUCTURE DUE TO PROCESSING VARIABLES

Hansen (1960) found that with increased chopping times the fat cells were broken much faster than the muscle fibers. The protein matrix was completely broken when chopped to 23.3°C, and 26.7°C resulted in breakage of the protein matrix, but no fat separation occurred. Helmer and Saffle (1963) used light microscopy to examine meat emulsions chopped to different temperatures. The fat droplets were small at 15.5°C but enlarged as temperature increased. At 26.7 and 32.2°C, the fat droplets were broken and formed large fat leaks. Jones and Mandigo (1982) reported that a thicker protein coating was deposited onto fat globules until a chopping temperature of 16°C was reached. The emulsion matrix was slightly eroded at 22°C and severely eroded at 28°C. Swasdee et al. (1982), in their study of changes during chopping and cooking of meat emulsions, suggested that after 15 minutes of chopping the batter became less heterogeneous, but did not become completely homogeneous. Collagen structures were severely altered, while muscle fibrils were altered to a lesser extent at an internal temperature of 70°C.

SALT LEVEL, CASING SIZE AND COOKING TEMPERATURE INFLUENCE ON BOLOGNA MICROSTRUCTURE

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SALT LEVEL, CASING SIZE AND COOKING TEMPERATURE INFLUENCE ON BOLOGNA MICROSTRUCTURE. Y. C. Wu, K. W. McMillin, J. S. Godber, and T. D. Bidner. Department of Animal Science, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

Beef bologna was manufactured with 0.75% or 2.25% salt, stuffed into 9.6 or 13.6 cm casings and cooked to 67 or 74°C. Evaluations of smokehouse yield, Instron shear value and scanning electron microscopy (SEM) microstructure were made. Bologna treatments with 2.25% salt, in 13.6 cm casings or with endpoint temperatures of 74°C resulted in greater ($p<0.05$) smokehouse yields and lower ($p<0.05$) shear values than for 0.75% salt levels, 9.6 cm casings or 67°C endpoint temperatures, respectively. SEM micrographs of cryofractured, Au/Pd-coated samples showed more uniform emulsion structures for 2.25% salt than for 0.75% salt treatments and indicated that less heat denaturation occurred in 13.6 cm casings than for bologna in 9.6 cm casings. The low smokehouse yield with 0.75% salt indicated that with decreased salt levels, processing variables must be closely monitored if conventional bologna characteristics are the desired result.

INTRODUCTION

The emulsion formation, structure and functional properties of comminuted sausages are influenced by many processing variables (Acton et al., 1983), but interactions of different conditions for gel matrix formation and stabilization have not been well characterized. Emulsion stability is influenced by many factors including the water binding capacity of the proteins, fat holding capacity of the protein matrix, the level of meat, water, fat, and nonmeat additives in the formulation, mechanical treatment and heat treatment (Schut, 1976). Reduction in the NaCl content of emulsion-type sausage causes a reduction of water binding capacity, emulsion stability and influences other functional properties (Offer and Trinick, 1983; Puolanne and Terrell, 1983; Sofos, 1983). Differences in muscle tissue microstructure caused by aging, enzyme action, and heating have been examined by scanning electron microscopy (SEM) (Schaller and Powrie, 1971; Eino and Stanley, 1973; Leander et al., 1980). There has been limited usage of SEM in the quality evaluation of processed meat products because a primary disadvantage of SEM has been in distinguishing fat from protein components in sausage microstructures (Ray et al., 1979). In the present study, bologna was manufactured with 0.75 or 2.25% salt 9.6 or 13.6

cm cellulose casings and 67 or 74°C endpoint smokehouse heating temperatures to examine differences in smokehouse yield, Instron shear values and microstructure of meat bologna.

MATERIALS AND METHODS

Ten-kg batches of bologna were prepared with lean beef trimmings and pork backfat. Unfrozen lean beef was ground through a 0.93-cm plate and preblended with 0.75% salt, held for 24 hours, then chopped in a Manurhin silent cutter, with 10% ice and either no additional salt or 1.5% percent salt, to a temperature of 4.5°C. Pork backfat (to comprise 30% of the meat block), spices and curing ingredients were added and chopping was continued until a temperature of 18.3°C was reached. Each of the eight meat emulsion batches (four replications of each salt level) was stuffed into both 9.6 and 13.6-cm cellulose casings. The two final endpoint temperatures (67°C and 74°C) were randomly assigned to each salt and casing treatment combination. Four smokehouse cycles of 20 min. at 57°C and 36.5% relative humidity (RH), 30 min. at 65.5°C and 56% RH and 93°C and 60% RH were used for pairs of 0.75% and 2.25% salt-level batches in each cycle until the proper internal endpoint temperatures of 67 and 74°C were attained. All bologna samples were stored in a 4°C cooler after smoking and cooking.

Weights of encased bologna were taken immediately before thermal processing and at 18 hours after chilling (4°C) for calculation of smokehouse yield as a percentage of initial encased weight. Proximate composition (moisture,

ether extract, Kjeldahl protein and ash) of finished bologna was determined on randomly selected bologna slices of each treatment combination (AOAC, 1980). Six core samples (1.2-cm diameter) were removed from a 0.6 cm thick slice of each treatment combination. Duplicate core samples were sheared by means of an Instron Universal Testing Machine (Model Number 1122, Warner-Bratzler shear type head) operated at a crosshead speed of 10 cm/min and a 10-kg full scale load range. Samples for scanning electron microscopy were prepared with a simple cryofracturing technique to reduce the disruption of protein-coated globules with complex chemical fixation procedures (Ray et al., 1979; Jones and Mandigo, 1982). A 5x5x10-mm section was cut with a razor blade from a randomly selected slice of each bologna treatment, treated in liquid Freon (-110^{o}C) for 30 seconds and then submerged and held in liquid nitrogen (-196^{o}C) until impact fracturing to expose the intended viewing surface. The frozen specimens were lyophilized in a cold vacuum chamber overnight, then mounted on 13-mm aluminum stubs with double stick Scotch^{R} cellophane tape. A thin (100 $\overset{o}{A}$) Au/Pd coat was applied onto the specimen with a diode sputtering unit (Hummer 1). Micrographs were taken on a Hitachi S-500 scanning electron microscope at an accelerating voltage of 25 KV.

A split plot design with four replications (smokehouse cycle) and the two salt levels within each smokehouse cycle as the main plot effects and with the combinations of casing

sizes and endpoint temperatures as split plot treatments was utilized (Snedecor and Cochran, 1980). Mean separation of smokehouse yield and Instron shear force values was accomplished by using least-squares differences when analysis of variance indicated significant treatment or interaction effects. The statistical model employed for analysis was:

$$Y = u + R + S + Ea + C + T + (C \times T) + (S \times C) + (S \times T) + (S \times C \times T) + Eb + Es;$$

where R = effect due to replications,

S = effect due to salt levels,

Ea = (R x S) error term for main plot,

C = effect due to casing sizes,

T = effect due to endpoint temperatures,

C x T = interaction between C and T,

S x C = interaction between S and C,

S x T = interaction between S and T,

S x C x T = interaction among S, C, and T,

Eb = R x C x T (S) error term for sub plot treatments,

Es = sampling error used to test Eb.

RESULTS AND DISCUSSION

The proximate analyses of finished bologna were not significantly different except for the effect of salt. Bologna treatments with 0.75 % salt had 60.3% moisture, 17.2% protein, 19.8% fat and 1.89% ash while bologna with 2.25% salt had 59.0% moisture, 13.1% protein, 24.4% fat and 2.64% ash.

The mean squares values from the analyses of variance for smokehouse yield and Instron shear values for the bologna treatments are shown in Appendix Table 1. Smokehouse yield was different ($p<0.05$) for the main effects of salt levels, casing sizes and endpoint cooking temperatures. There were also highly significant ($p<0.01$) differences in smokehouse yield due to the two-way interactions among the main treatment effects.

The 2.25% level of salt produced greater ($p<0.05$) smokehouse yields than the 0.75% level of salt (Table 1). This is in agreement with Sofos (1983) who reported that smokehouse yield of frankfurters decreased as the amount of salt decreased. The decreased smokehouse yield in the present study indicated that the lower level of salt contributed to a less stable emulsion structure during formation or thermal processing than did the conventional (2.25%) salt level and allowed more loss of fat from the

emulsion structure. The smokehouse yield did not vary significantly with casing size or endpoint cooking temperature when bologna was produced with 2.25% salt. Lee et al. (1981) found that a higher cooking temperature (70°C) resulted in lower retention of fat and water than a lower (60°C) cooking temperature and attributed this to a more rapid heating rate at 70°C and a slower heating rate at 60°C. In the present study, the smaller casing treatments were held in the smokehouse until the 13.6-cm casing treatments reached the desired internal endpoint temperatures. The heating rate for bologna in the 9.6-cm casings was slightly faster than for the bologna in 13.6-cm casings and so the smokehouse yields with each of the casing sizes were slightly, but not significantly, lower for bologna containing 2.25% salt cooked to 67°C rather than 74°C. The present results with 2.25% salt levels did not support the findings of Mittal and Blaisdell (1983) that moisture loss rate was proportional to product temperature, but with 0.75% levels of salt, the 67°C endpoint temperatures did result in greater smokehouse yields than the 74°C temperature with both casing sizes. These conflicting results with the two different salt levels are the cause of significant interaction effects. Of the 0.75% salt treatments, only bologna produced in 13.6-cm casing and heated to 67°C had smokehouse yields similar to the bologna manufactured with 2.25% salt.

Instron shear values, expressed as the kg of force per

cm^2 that was required to shear through the bologna slice, varied ($p<0.05$) with the level of salt and size of casing, but not with endpoint temperature (Table 1). However, the interaction of temperature and casing size and the three way interaction were highly significant ($p<0.01$). It can be seen that Instron shear values did not differ greatly between the two salt levels for a given casing size and temperature combination except for the 67°C and 13.6-cm casings where treatments with 0.75% salt required more force ($p<0.05$) for shearing than 2.25% salt levels. Sofos (1983) found that the shear force required to shear intact products (frankfurters) decreased with decreased salt levels. There was a trend in the present study for bologna encased in 9.6-cm casings to have lower shear values than for bologna stuffed into 13.6-cm casings and for bologna cooked to 67°C to have lower shear values than when bologna was thermally processed to 74°C. The proportions of myofibrillar protein solubilization and conversion of collagen to gelatin were not measured, but the additional heating of 9.6-cm encased bologna, which was held in the smokehouse until the 13.6-cm bologna reached the desired endpoint temperature, may have caused more gelatin formation than in 13.6-cm casings. The higher internal temperature of 74°C would cause more myofibrillar coagulation than cooking to 67°C. This would explain the contradiction due to heating in the shear force measurements. It was observed that the bologna with 0.75% salt was softer in texture than bologna

produced with the higher (2.25%) level of salt.

Micrograph 1A is an example of the scanning electron micrographs of a bologna sample after the cryofracturing technique. A matrix network can be observed (M), which depicts an example of the protein found in the continuous phase of the meat emulsion as described by Ray et al. (1979). An intact muscle fiber (F) and a protein encapsulated fat globule (G) can also be identified. Micrograph 1B reveals a 2,000X magnification of a stable protein-encapsulated fat globule. The high salt (2.25%) groups had a homogeneous meat emulsion as shown in Micrographs 2A (500X) and 2B (1,500X). The fat globules were tightly bound by the protein matrix as shown in 2B. The 0.75% salt treatments had a much more loosely bound emulsion structure as shown in Micrographs 3A (500X) and 3B (1,500X). The 0.75% salt treatment had less batter expansion and more batter contraction as has been indicated by Prusa et al. (1985) where the addition of sodium chloride increased batter expansion and decreased batter contraction. The contraction of the low salt batter may be responsible for the loose structure of the meat emulsion. This difference in structure between 2.25 and 0.75% salt levels would explain the lower smokehouse yields observed in this study. There was not enough myofibrillar or salt-soluble protein extracted with 0.75% salt to encapsulate the fat globules and form the stable gel matrix during thermal processing that was observed in bologna made with the additional 1.5%.

Bologna encased in 13.6-cm casings appeared to have less heat denaturation of proteins than bologna in 9.6-cm casings (Micrographs 4A and 4B). The visible indicators of heat denaturation may be observed in the micrograph of bologna thermally processed in smaller casings 9.6 cm (Micrograph 4B), but were not evident for treatments of bologna encased in 13.6-cm casings (Micrograph 4A). Similar heat denaturation patterns between bologna cooked to 67°C or 74°C are apparent in Micrographs 5A and 5B. This may explain the differences in smokehouse yields and shear force values for the two endpoint cooking temperatures.

Food processors and consumers are concerned about the sodium levels in processed foods, but salt is necessary for protein solubilization and the subsequent proper sausage emulsion matrix formation in finely comminuted sausages. The present research showed that texture, smokehouse yields and gel-matrix structures of processed sausages with reduced levels of salt are influenced by casing size and cooking procedures more than when finely-comminuted sausages are prepared with more traditional levels of salt. Sausage manufacturers who lower the salt content of their comminuted meat products must closely monitor the production practices for each product type, casing size and cooking procedure if those products are to have similar characteristics to sausages that are manufactured with normal salt levels.

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Table 1 - Effect of salt level, casing size, and endpoint cooking temperature on smokehouse yields and Instron shear values of bologna

Treatments Salt (%)	Casing Size (cm)	Temp. (°C)	n[a]	Smokehouse Yield %[b]	Instron Shear Value, kg/cm^2
2.25	9.6	67	8	94.58^{d}	2.18^{f}
		74	8	94.78^{d}	2.78^{f}
	13.6	67	8	94.03^{d}	3.29^{e}
		74	8	94.83^{d}	3.55^{e}
0.75	9.6	67	8	90.15^{ef}	2.33^{ef}
		74	8	81.35^{g}	2.82^{ef}
	13.6	67	8	93.08^{de}	5.25^{d}
		74	8	88.73^{f}	3.30^{e}
		S.E.[c]		1.32	0.33

[a]Number of samples of each treatment combination.

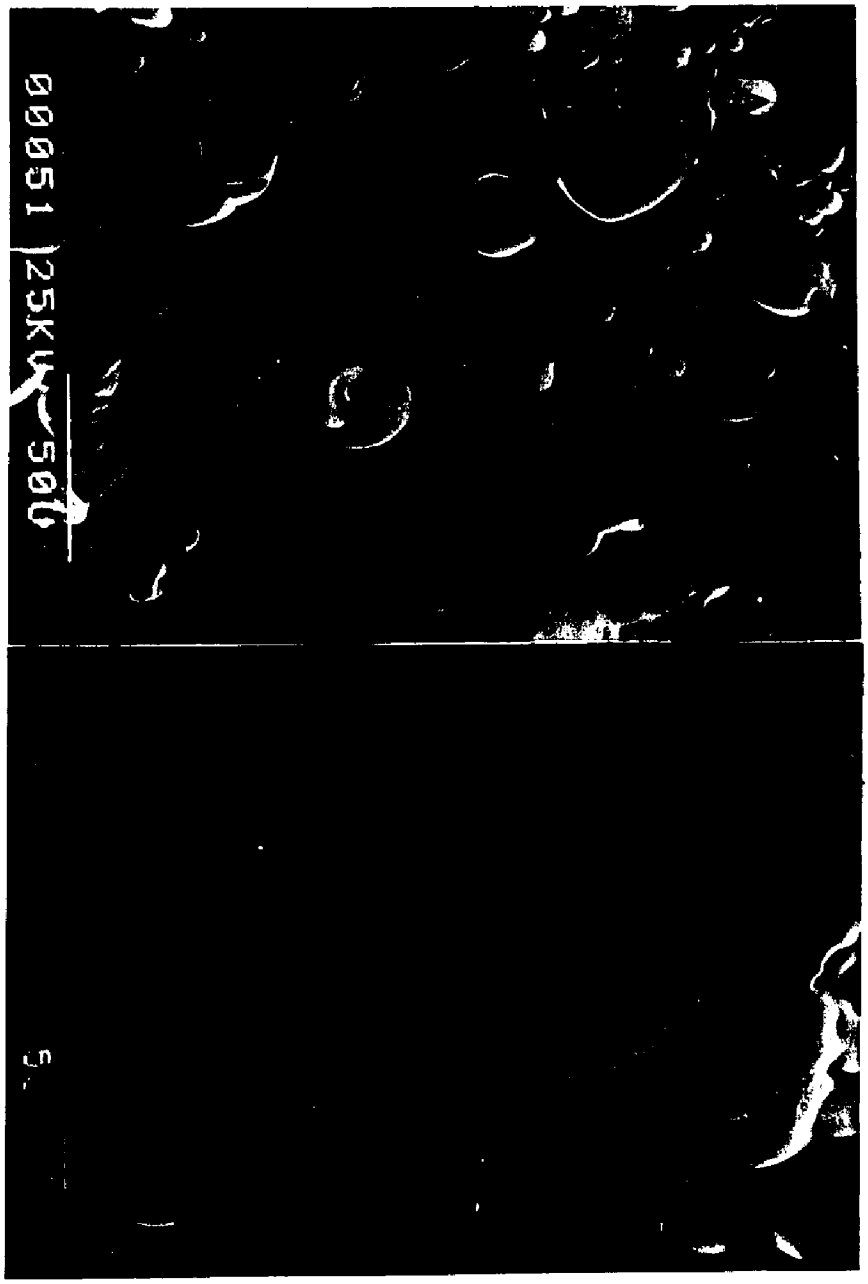
[b]Smokehouse Yield = weight of bologna before thermal processing/chilled weight x 100%.

[c]Standard error of least square means.

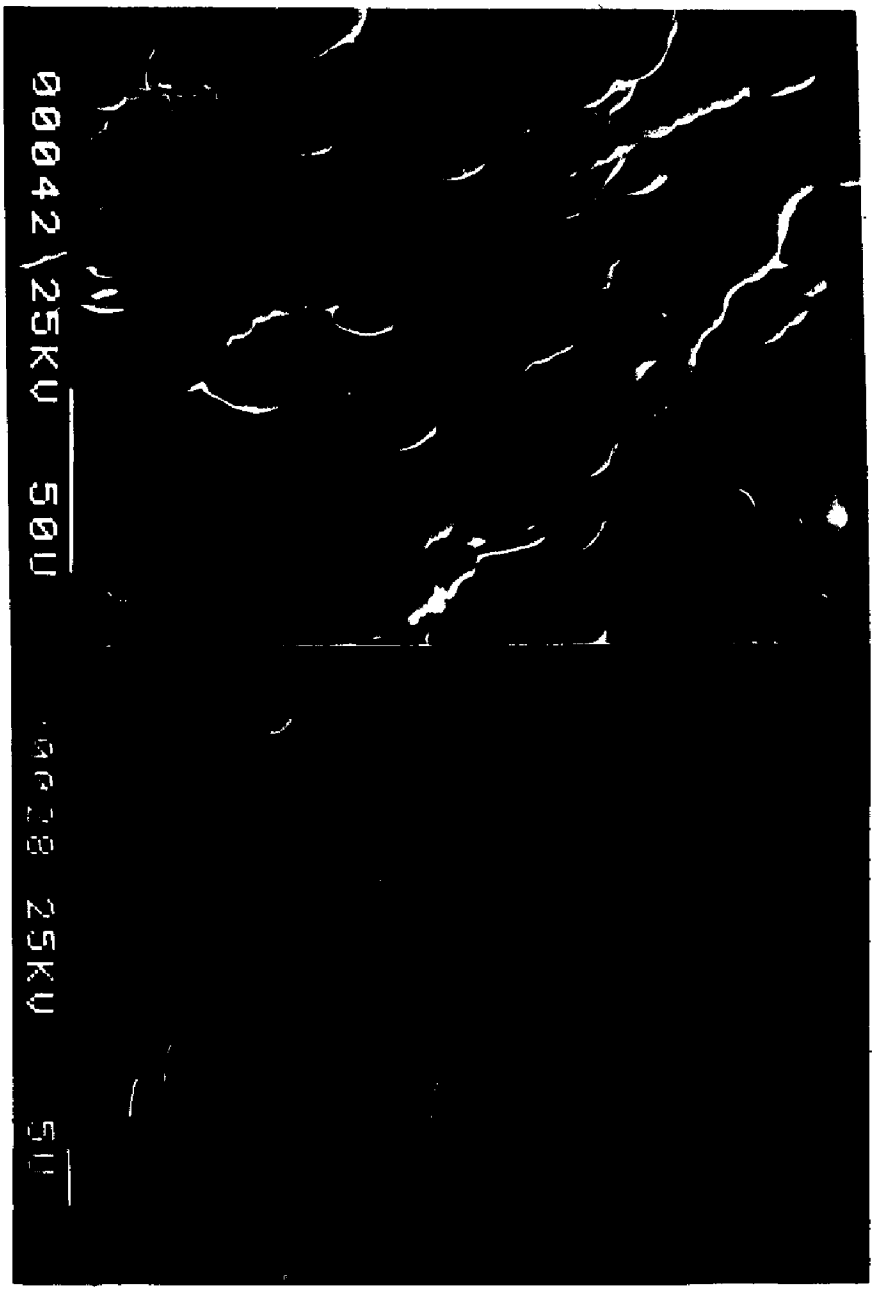
[defg]Least square means in the same column with the same superscript are not different ($p>0.05$).

Micrograph 1 - SEM micrographs of bologna. A. 500X. B. 2,000 X. F: Intact muscle fiber. M: Emulsion matrix. G: Encapsulated fat globule.

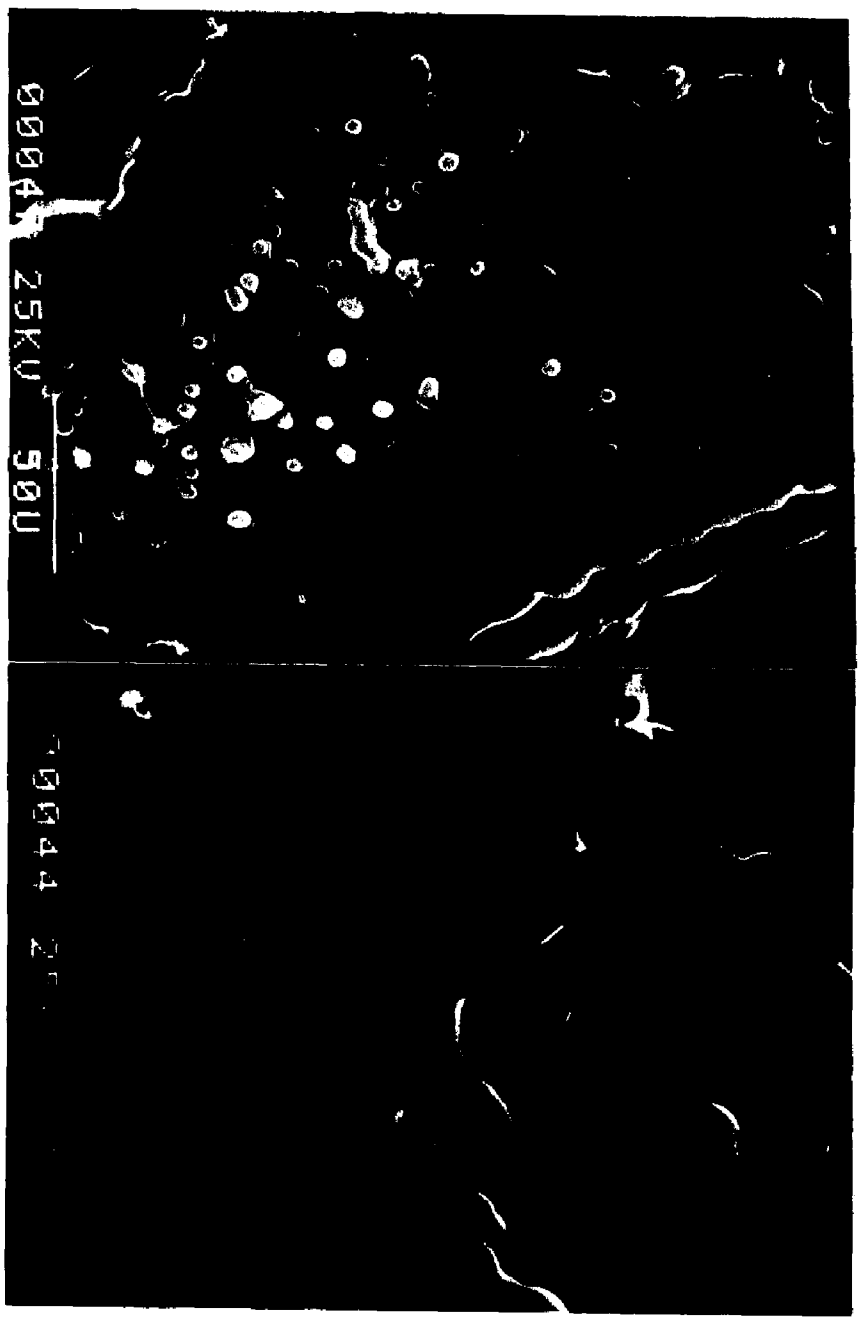
Micrograph 2 - Effect of salt level on microstructure of bologna cooked to 67°C and encased in 13.6 cm casing. A. 2.25% salt (500X). B. 2.25% (1,500X).


00051 25KV 500

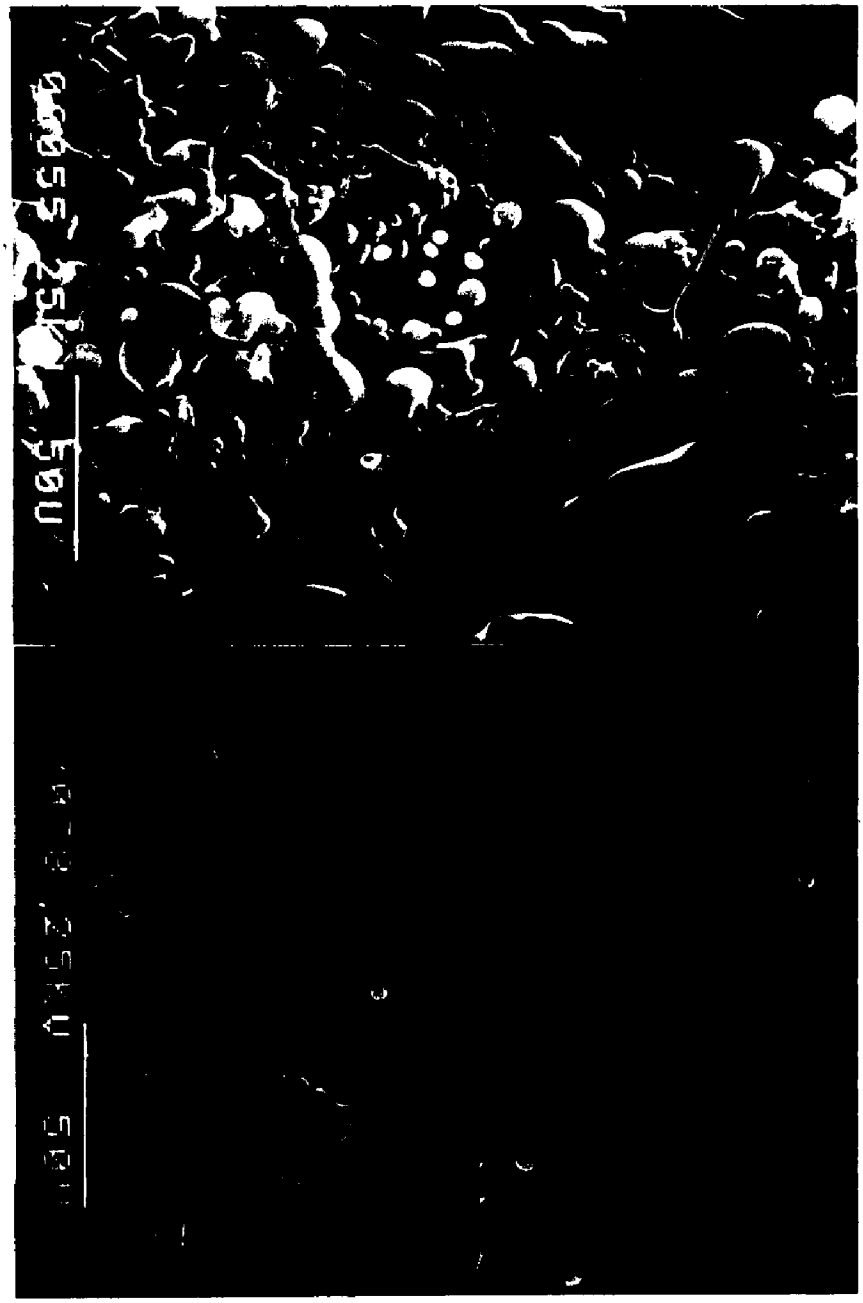
Micrograph 3 - Effect of salt level on microstructure of bologna cooked to 67°C and encased in 13.6 cm casing. A. 0.75% salt (500X). B. 0.75% salt (1,500X).


00042 25KV 50U
00018 25KV 5U

Micrograph 4 - Effect of casing size on microstructure of bologna with 2.25% salt and cooked to 67°C. A. 13.6 cm casing (500X). B. 9.6 cm casing (500X).


00045 25KV 500U
00044 25

Micrograph 5 - Effect of final endpoint cooking temperature on microstructure of beef bologna with 2.25% salt and encased in 9.6 cm casing. A. 67°C (500X). B. 74°C (500X).



INFLUENCE OF BEEF SOURCE, SALT, CASING SIZE AND COOKING TEMPERATURE UPON BOLOGNA PROPERTIES AND MICROSTRUCTURE

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INFLUENCE OF BEEF SOURCE, SALT, CASING SIZE AND COOKING TEMPERATURE UPON BOLOGNA PROPERTIES AND MICROSTRUCTURE.

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Bologna was manufactured with beef from forage- or grain- finished steers, pork backfat, 1, 2 or 3% salt, chopped to 10, 15.5, and 21°C, stuffed into 9.6 or 13.6 cm casings and cooked to 67 or 74°C for evaluation of water binding capacity and emulsion stability in raw emulsions and smokehouse yield, Instron shear value and scanning electron microscopy (SEM) microstructure in final products. Bologna from forage-finished beef had lower ($p<0.05$) water, oil and total losses and a higher ($p<0.05$) smokehouse yield than bologna from grain-fed beef except for the 3% salt level. Treatments with higher salt levels had lower total oil and water losses and higher water binding capacities. Emulsion stability tended to increase as chopping temperature increased in the 3% salt group. Samples with 3% salt had higher smokehouse yields and did not differ in yields with different endpoint temperatures. Samples with 1 or 2% salt had a higher smokehouse yield with 67°C endpoint temperature than with 74°C endpoint temperature. SEM micrographs showed that the 3% level of salt treatment had a thicker protein coating and more stable emulsion as compared

to 1 or 2% salt treatments. Less heat denaturation occurred for bologna in 13.6 cm casings than in 9.6 cm casings. Salt levels lower than 3% resulted in decreased smokehouse yield, water binding capacity, and emulsion stability. Meat processors need to consider alternative processing techniques when salt levels are reduced if the bologna are to have the desired physical characteristics.

INTRODUCTION

The level of salt in processed meat products has been of concern since dietary sodium has been linked to hypertension in humans (Sofos, 1983; Hand et al., 1982). Salt in processed meats greatly influences product flavor, texture and extractability of the salt-soluble proteins that bind fat, moisture and other ingredients (Hamm, 1960; Saffle, 1968; Theno, 1978). Several approaches have been used to decrease the sodium levels, including reducing the NaCl content, replacing part of the NaCl with other chloride salts or nonchloride-salt compounds, and altering processing techniques (Terrell, 1983). Reduction in the NaCl content of emulsion-type sausages will decrease water-binding capacity, emulsion stability and other sausage functional properties (Offer and Trinick, 1983; Puolanne and Terrell, 1983; Sofos, 1983; Whiting, 1984). The emulsion stability and structural and functional properties of comminuted sausages are influenced by individual processing variables such as salt levels, chopping temperatures, fat levels, humidity levels and cooking temperatures (Helmer and saffle, 1963; Townsend et al., 1971; Acton et al., 1983). However, the interactions of different processing conditions and sources of meat for gel matrix formation and meat emulsion stabilization have not been well characterized. Williams et

al. (1983) found differences in forage-finished and grain-finished beef composition. Costello et al. (1985) reported that increased days on feed decreased the total cooking losses and Warner-Bratzler shear force in restructured beef steaks, but there are no reports of influences by lean meat from different dietary sources on meat emulsion characteristics.

Scanning electron microscopy (SEM) has shown a wide variation in the microstructure among different frankfurters (Cassens et al., 1975). However, Theno and Schmidt (1978) published SEM micrographs of meat emulsions which showed that fat globules were entrapped within a lace-like or lattice structure of protein filaments in commercial frankfurters. Swasdee et al. (1982) suggested that frankfurters prepared by conventional chopping methods were still a heterogeneous, multiphase system and heating caused more structural changes than chopping. Jones and Mandigo (1982) published SEM micrographs which indicated that rupturing of encapsulated fat globules increased as chopping temperature increased.

In the present study, bologna was manufactured with two sources of lean and three salt concentrations, encased in two sizes of cellulose casings, and heat processed to two endpoint smokehouse heating temperatures. The major purpose of this study was to assess bologna stability and examine the microstructure to further elucidate differences due to processing conditions.

MATERIALS AND METHODS

Ten-kg batches of bologna were prepared with lean beef chuck trimmings from either grain-finished or forage-finished cattle and pork backfat. Unfrozen lean beef was ground through a 0.93-cm plate and preblended with 1% salt, held for 24 hours, then chopped in a Hobart silent cutter (1,500 rpm) with 10% ice and 0, 1%, or 2% additional salt to a temperature of 4.5°C. Pork backfat (to comprise 26% of the meat block), spices and curing ingredients were added and chopping was continued to 18.3°C. Samples were taken at 10, 15.5, and 21.1°C chopping temperatures to determine the emulsion stability and water holding capacity. Each of the six meat emulsion batches (two replications of each salt level) was stuffed into both 9.6 and 13.6-cm cellulose casings. The two final endpoint temperatures (67°C and 74°C) were randomly assigned to each salt and casing treatment combination. Similar smokehouse cycles (20 min. at 56°C and 36.5% relative humidity (RH), 30 min. at 65.5°C and 56% RH, and 93°C and 60% RH to the desired internal temperature) were used for all treatments until the proper internal endpoint temperatures of 67 or 76°C were obtained. The lean beef used in this study was from steers finished either on forage only or 108 days corn silage plus added corn. Age at slaughter, backfat thickness and quality grade

were, respectively, 23 months, 0.33 to 0.58 cm and low good to low choice for forage-finished steers and 20 months, 0.66 to 1.25 cm and high good to high choice for grain-finished steers.

Weights of bologna were taken immediately before thermal processing and at 18 hours after 4° chilling for calculation of smokehouse yields as percentages of initial weight. Proximate composition (moisture, ether extract, Kjeldahl protein and ash) of raw emulsions and finished bologna was evaluated on randomly selected bologna slices for each treatment within each batch using AOAC (1980) procedures. Emulsion stability (Rongey, 1965) was determined using 25 g of raw emulsion samples to determine percent oil (fat) loss (ESO), percent water loss (ESW) and percent total loss (EST). Water binding capacity was determined using the method developed by Wierbicki (1959). A small amount of emulsion sample placed on a piece of filter paper between two plexiglass plates was submitted to 3,000 psi pressure for 3 minutes. Then the total area and meat area was measured with a planimeter. The ratio of total area and meat area as measured with a planimeter was taken as a relative measure of water binding capacity. Six core samples (1.2-cm diameter) were removed from a 0.6 cm thick bologna slice of each treatment combination. The core samples were sheared in duplicate with an Instron Universal Testing Machine (Model Number 1122, Warner-Bratzler shear type head) operated at a crosshead speed of 10 cm/min and a

10 kg full scale load range.

Two slices of bologna were randomly selected from each treatment for SEM evaluation. Cubes of 1.5 mm x 1.5 mm were cut from each sample (4°C) with a chilled razor blade. Each cube was treated in liquid freon (-110°C) for 30 seconds and then submerged and held in liquid nitrogen (-196°C) until impact fracturing exposed the viewing surface. After removal from the liquid nitrogen, the frozen samples were fixed in 3% glutaraldehyde fixative buffered with 20mM MES [2(N-morpholino) ethane sulfonic acid], for 18 hours at 4°C. All specimens were then post-fixed for 30 min. in a 1% osmium tetroxide solution to stabilize the lipid. After fixation, the samples were rinsed twice in 20mM MES for 5 min. Specimen dehydration in graded ethanol solutions of 25, 50, 75, 90, 100, 100, and 100% for 10 min. each at 23°C was followed by vacuum desiccation with running water aspiration for 3 hours as described by Basgall et al. (1983). The samples were then mounted on 13-mm aluminum stubs with double-stick Scotch^{R} cellophane tape. A thin (100 Å) Au/Pd coat was applied onto the specimen with a diode sputtering unit (Hummer I). Micrographs were taken on a Hitachi S-500 scanning electron microscope at an accelerating voltage of 25 KV.

A 2x3x2x2 completely randomized design with two replications of each treatment combination was analyzed using diets, salt levels, casing sizes and endpoint temperatures as the main effects in analysis of variance

(Snedecor and Cochran, 1980) for proximate analysis of finished bologna, smokehouse yield and Instron shear force. The statistical model was :

$$Y = u + D + S + C + E + (D \times S) + (D \times C) + (D \times E) + (S \times C) + (S \times E) + (C \times E) + (D \times S \times C) + (D \times C \times E) + (S \times C \times E) + e;$$

where D = effect due to diets,

S = effect due to salt levels,

C = effect due to casing sizes,

E = effect due to endpoint temperatures,

D x S = interaction between diets and salt levels,

D x C = interaction between diets and casing sizes,

D x E = interaction between diets and endpoint temperatures,

S x C = interaction between salt levels and casing sizes,

S x E = interaction between salt levels and endpoint temperatures,

C x E = interaction between casing sizes and endpoint temperatures,

D x S x C = three way interaction among diets, salt levels, and casing sizes,

D x C x E = three way interaction among diets, casing sizes, and endpoint temperatures,

S x C x E = three way interaction among salt levels, casing sizes, and endpoint temperatures,

e = random error.

A 2 x 3 x 2 completely randomized design with two replications of each treatment combination was analyzed using diets, salt levels, and chopping temperatures as the main effects in the analysis of variance for proximate analysis (raw emulsions), emulsion stability and water binding capacity. The statistical model was:

$$Y = u + D + S + T + (D \times S) + (D \times T) + (S \times T) + (D \times S \times T) + e;$$

where D = effect due to diets,

S = effect due to salt levels,

T = effect due to chopping temperatures,

D x S = interaction between diets and salt levels,

D x T = interaction between diets and chopping temperature,

S x T = interaction between salt levels and chopping temperatures,

D x S x T = three way interaction among diets, salt levels, and chopping temperatures,

e = random error.

Least square differences were obtained for individual mean separations when analysis of variance indicated significant treatment effects (SAS, 1979).

RESULTS AND DISCUSSION

Proximate composition of raw emulsions is shown in Table 2. Ash content was different ($p<0.05$) for the main effect of salt levels. In the grain-fed group, moisture and protein content were higher ($p<0.05$) with 1% salt level than with 2 and 3% salt levels. Decreased moisture and increased protein contents were observed in 1 and 2% salt treatments of the grass-fed group compared to the 1 and 2% salt treatments of the grain-fed group. The fat content of each batch was adjusted to the same amount (26% of the meat block) for all treatments in this experiment. However, Williams et al. (1983) indicated that forage-finished beef had a higher protein content (18.1%) and lower fat content (19.1%) compared to grain-finished beef with 15.4% protein and 34.1% fat.

The treatments with higher salt levels had lower ($P<0.05$) total oil and water losses (Table 3). This is in agreement with Sofos (1983) who stated that emulsion stability increased as the amount of salt increased. Increased salt levels caused increased ($P<0.05$) water binding capacity. This agreed with the data of Ockerman (1980) that meat with 3% added salt had higher water binding capacity than meat with 2% added salt. Emulsion stability and water binding capacity increased as chopping temperature

increased among all salt levels. However, no difference was found between 15.5 and 21°C chopping temperature in 1 and 3% salt levels. With the 1% salt level, there was not enough salt-soluble protein to stabilize the emulsion; therefore, increases in the chopping temperature alone did not affect water and oil binding. Sofos (1983) stated that 2% NaCl is required to produce a stable meat-emulsion without any other substitution. With 3% salt, a relatively low chopping temperature (15.5°C) was enough to achieve maximum binding and a higher chopping temperature did not improve emulsion stability. With the 2% salt level, water binding capacity and emulsion stability increased as the chopping temperature increased. This is in agreement with Brown and Toledo (1975) who stated that the temperature range of maximum binding in meat batters was 15 to 22°C.

Water binding capacity, emulsion stability and smokehouse yields increased with increased salt levels with both types of lean (Table 4). Salt levels of 1 and 2% in grass-fed treatments showed lower ($p<0.05$) free water, oil and total losses and higher ($p<0.05$) smokehouse yields than corresponding salt levels in grain-fed treatments. The higher protein content in the grass-fed group might be responsible for these results, causing a greater water binding capacity and fat binding capacity. Christian and Saffle (1967) stated that more highly unsaturated fatty acids were less emulsified than saturated fatty acids. Williams et al. (1983) found that forage-finished beef

contained a lower % of total unsaturated fatty acid (47.7%) than grain-finished beef (53.1%). They also reported that forage-finished beef contained a higher polar fat content (4.6%) than grain-finished beef (1.2%). In the present study, emulsions from the forage-fed beef showed a higher emulsion stability compared to beef finished on grain. Salt is not soluble in nonpolar fat (Trout and Schmidt, 1986), so the effective salt concentration would be expected to be slightly greater in emulsions from forage-finished beef than from grain-finished beef, increasing the emulsion capacity and stability. In general, the grass-fed group had higher ($p<0.05$) smokehouse yields than grain-fed group. However, no differences were found at the 3 % salt level between the two diet groups.

Proximate composition of bologna samples is shown in Table 5. Samples with a 3% level of salt had higher ($p<0.05$) moisture contents and lower fat and protein contents than the 1 and 2% level of salt. Higher moisture content and lower protein were also observed in the grass-fed groups of bologna compared to grain-fed groups of bologna at most salt levels (Table 5). Ash content was different ($p<0.05$) for the main effect of salt levels.

Smokehouse yields were different ($p<0.05$) for the main effects of salt levels, casing sizes and endpoint cooking temperatures. Least square means were used to further determine differences among the twelve treatment combinations (Table 6). It can be seen that the 3% level

of salt produced greater ($p<0.05$) smokehouse yields than 1 and 2% level of salt in bologna. This is in agreement with Sofos (1983) who reported that smokehouse yields of frankfurters decreased as the amount of salt decreased. The decreased smokehouse yields indicated that the lower levels of salt contributed less to the emulsion structure during matrix formation or thermal processing than did the conventional salt level (3%). The smokehouse yields varied ($p<0.05$) with casing size or endpoint cooking temperature when bologna was produced with 1 and 2 % salt (Table 6). Lee et al. (1981) found that higher cooking temperatures ($70^{o}C$) resulted in lower retentions of fat and water than lower ($60^{o}C$) cooking temperatures and attributed this to a more rapid heating rate at $70^{o}C$ and a slower heating rate of $60^{o}C$. In the present study, 9.6-cm casing treatments had lower ($p<0.05$) smokehouse yields than the 13.6-cm casing treatments. The heating rate for bologna in the 9.6-cm casings was slightly faster than for the bologna in 13.6-cm casings. Moisture loss at the 3% salt levels did not agree with the findings of Mittal and Blaisdell (1983) who reported that the rate of moisture loss was proportional to product temperature. At the 1 and 2% levels of salt, the $67^{o}C$ endpoint temperatures did result in greater smokehouse yields with both casing sizes than the $74^{o}C$ temperature. Samples produced with a level of 3% levels of salt would be expected to have more salt soluble protein extracted and therefore, be more stable during cooking, thus showing less

difference in smokehouse yields. Trout and Schmidt (1986) found that increased effective salt concentrations increased the temperature to which the meat protein could be heated before aggregation. Increased salt would allow the meat protein matrix to hold more water and decrease the amount of water loss during thermal processing, as shown in the present study.

Instron shear values, expressed as the kg of force per cm^2 of required to cut through the bologna slice, varied ($p<0.05$) with the level of salt but not with the size of casing and endpoint temperatures. There was a trend for bologna encased in 9.6-cm casings to have higher shear values than for bologna stuffed into 13.6-cm casings (Table 6). The proportions of myofibrillar protein solubilization and conversion of collagen to gelatin were not measured, but 9.6-cm encased bologna may have formed more gelatin than the bologna in 13.6-cm casings. Bologna samples with 3% salt required less ($p<0.05$) force for shearing than 1 and 2% salt treatments. It was observed that bologna with 1 and 2% salt was softer in texture than bologna produced with the 3% salt. Sofos (1983) reported that force required to shear intact (skin-on) frankfurter products decreased with decreased salt levels. However, in the treatments with presalted ground chick meat, 1.5% salt treatments had higher shear forces than the 2.5% salt treatments at two and three weeks of storage. In the present study, the W-B shear head used might have compressed the samples before shearing more

for the samples of bologna with low salt than for the bologna with 3% salt levels.

The major difference in appearance of comminuted meat products is due to the mechanical treatment applied (salt level, casing size, and cooking temperature) during comminution (Schut, 1976; Schmidt et al., 1981; Jones and Mandigo, 1982). It was very difficult to distinguish any microstructural differences between the two diet group treatments; therefore, only micrographs of bologna from the grain-fed group were used for comparison of major treatment effects (salt, casing, and temperature).

A matrix network can be observed through SEM micrographs which depicts an example of the protein found in the continuous phase of the meat emulsion as described by Ray et al. (1981). The encapsulated fat globule is approximately 35 to 80 um in diameter under SEM. Some small pores were found on the surface of protein encapsulated fat globules. These pores might serve as a pressure-release type mechanism to stabilize the fat globule during cooking (Jones and Mandigo, 1982).

Distinct differences between salt treatments were observed in the fractured surface microstructures. The 3% salt treatments exhibited a homogeneous meat emulsion as shown in Micrographs 6A (500X) and 6B (2,000X). The fat globules were tightly bound by the protein matrix. The 1% salt treatments had a much more loosely bound emulsion structure as shown in Micrographs 8A (500X) and 8B (2,000X).

The 2% treatments showed 7A (500X) and 7B (2,000X) structure somewhat intermediate between 3 and 1% treatments. The 1% salt treatment appeared smoother and the surface structure resulting from the 3% salt treatments appeared rougher than the 2% salt treatments. It would be assumed that a thicker protein coating surrounded the fat globules in the 3% salt treatment (Jones and Mandigo, 1982). The matrix structures appeared to be more irregular, reflecting an increased extraction of myofibrillar proteins with increased level of salt. This difference in structure among 1, 2 and 3% salt levels would substantiate the lower smokehouse yields observed in this study. There was not enough myofibrillar or salt-soluble protein extracted with 1% salt to encapsulate the fat globules and form the stable gel matrix during thermal processing that was observed with bologna made with 2 or 3% salt.

Bologna encased in 13.6-cm casings appeared to receive less heat denaturation of proteins than bologna in 9.6-cm casings (Micrographs 9A and 9B). Heat denaturation may be shown through a globule clumping or coating of protein (Leander et al., 1980). These visible indicators of heat denaturation may be observed in the micrograph of bologna thermally processed in small casings (9.6-cm, Micrograph 9B) but were not evident for treatments of bologna encased in 13.6-cm casings (Micrograph 9A). Micrograph 10A (500X) and Micrograph 10B (2,000X) revealed the microstructure of bologna cooked to 67^{o}C and Micrograph 11A (500X) and

Micrograph 11B (2,000X) showed the microstructure of bologna cooked to 74°C. There did not appear to be gross structural differences between the two treatments; however, bologna cooked to 74°C seemed to cause more disruption in the protein matrix structure and more heat denaturation.

Food processors and consumers are concerned about the sodium levels in processed foods, but salt is necessary for protein solubilization and the subsequent proper sausage emulsion matrix formation in finely comminuted sausages. The present research showed that texture, smokehouse yields and gel-matrix structures of processed sausages with reduced levels of salt will be influenced by casing size and cooking procedures more than when finely comminuted sausages are prepared with more traditional levels of salt. Larger casing sizes and lower final temperatures (or slower heating rates) are recommended with 1 or 2% salt bologna production. Each phase of production must be closely monitored and the thermal processing matched to the desired casing size and product type when sausage manufacturers reduce the salt content of comminuted meat products.

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Table 2 - Effect of diet and salt level on proximate composition of raw emulsions.

Treatments Diet	Salt %	Moisture %	Crude Fat %	Protein %	Ash %
Grass	1	67.54^{a}	16.75^{bc}	14.41^{bc}	1.28^{a}
	2	67.09^{a}	16.86^{bc}	15.44^{c}	1.88^{b}
	3	68.85^{ab}	15.46^{a}	12.66^{ab}	3.02^{c}
Grain	1	70.53^{b}	14.55^{a}	13.64^{b}	1.19^{a}
	2	68.89^{ab}	17.40^{c}	11.90^{a}	1.79^{b}
	3	67.84^{a}	16.37^{b}	12.92^{ab}	2.85^{c}
	SE	.16	.15	.04	.04

^{abc} Least square means in the same column with the same superscript are not different ($p>0.05$).

Table 3 - Effect of salt level and chopping temperature on water binding capacity and emulsion stability of raw emulsions.

Treatments Salt %	Temp. °C	WBC[a] %	ESW[b] %	ESO[b] %	EST[b] %
1	10	25.59^{i}	31.25^{i}	6.63^{h}	37.88^{j}
1	15.5	19.74^{h}	28.38^{hi}	5.75^{gh}	34.13^{i}
1	21	19.38^{h}	29.38^{hi}	5.00^{g}	34.38^{i}
2	10	11.56^{g}	22.88^{g}	4.82^{g}	27.69^{h}
2	15.5	11.19^{g}	21.00^{f}	3.38^{ef}	24.38^{g}
2	21	6.31^{e}	18.88^{e}	2.63^{e}	21.50^{f}
3	10	3.30^{de}	10.13^{de}	2.37^{de}	12.50^{e}
3	15.5	1.82^{d}	8.75^{d}	1.62^{d}	10.38^{d}
3	21	$.92^{d}$	7.88^{d}	1.00^{d}	8.88^{d}
	SE[c]	.29	.43	.20	.54

[a]WBC = water binding capacity. % of water loss as determined by press method.

[b]ESW = % of water loss, ESO = % of oil loss and EST = % of total water and oil loss from 25 g of raw emulsion sample.

[c]SE = standard error of the least squares means.

defghij Least squares means in the same column with the same superscript are not different ($p>0.05$).

Table 4 - Effect of diet and salt level on water binding capacity, emulsion stability and smokehouse yields of bologna.

Treatments Diet	Salt %	WBC %[a]	ESW %[b]	ESO %[b]	EST %[b]	Yield %[c]
Grass	1	14.68[g]	23.25[g]	3.25[f]	26.50[g]	74.82[g]
	2	5.71[f]	18.92[f]	3.33[f]	22.25[f]	79.78[h]
	3	1.38[e]	9.58[e]	1.50[e]	11.08[e]	86.46[i]
Grain	1	28.46[h]	36.08[h]	8.33[g]	44.41[h]	65.35[e]
	2	13.67[g]	22.91[g]	3.87[f]	26.79[g]	68.75[f]
	3	2.67[e]	8.25[e]	1.83[e]	10.08[e]	83.65[i]
	SE[d]	.24	.35	.16	.44	.49

[a]WBC = water binding capacity. % of water loss as determined by press method.

[b]ESW = % of water loss, ESO = % of oil loss and EST = % of total water and oil loss from 25 g of raw emulsion sample.

[c]Yield = Weight of bologna before thermal processing / chilled weight x 100%.

[d]SE = standard error of the least squares means.

efghi Least squares means in the same column with the same superscript are not different ($p>0.05$).

Table 5 - Effect of diet and salt level on proximate composition of bologna.

Treatments Diet	Salt %	Moisture %	Crude Fat %	Protein %	Ash %
Grass	1	60.18^{bc}	22.14^{bc}	16.08^{ab}	1.56^{a}
	2	59.73^{b}	22.63^{bc}	15.34^{a}	2.28^{b}
	3	61.89^{c}	19.65^{a}	15.26^{a}	3.12^{c}
Grain	1	57.13^{a}	21.70^{ab}	19.64^{c}	1.53^{a}
	2	57.05^{a}	23.20^{c}	17.67^{b}	2.15^{b}
	3	60.59^{bc}	20.92^{a}	15.30^{a}	3.03^{c}
	SE	.29	.19	.17	.07

abc Least squares means in the same column with the same superscript are not different ($p>0.05$).

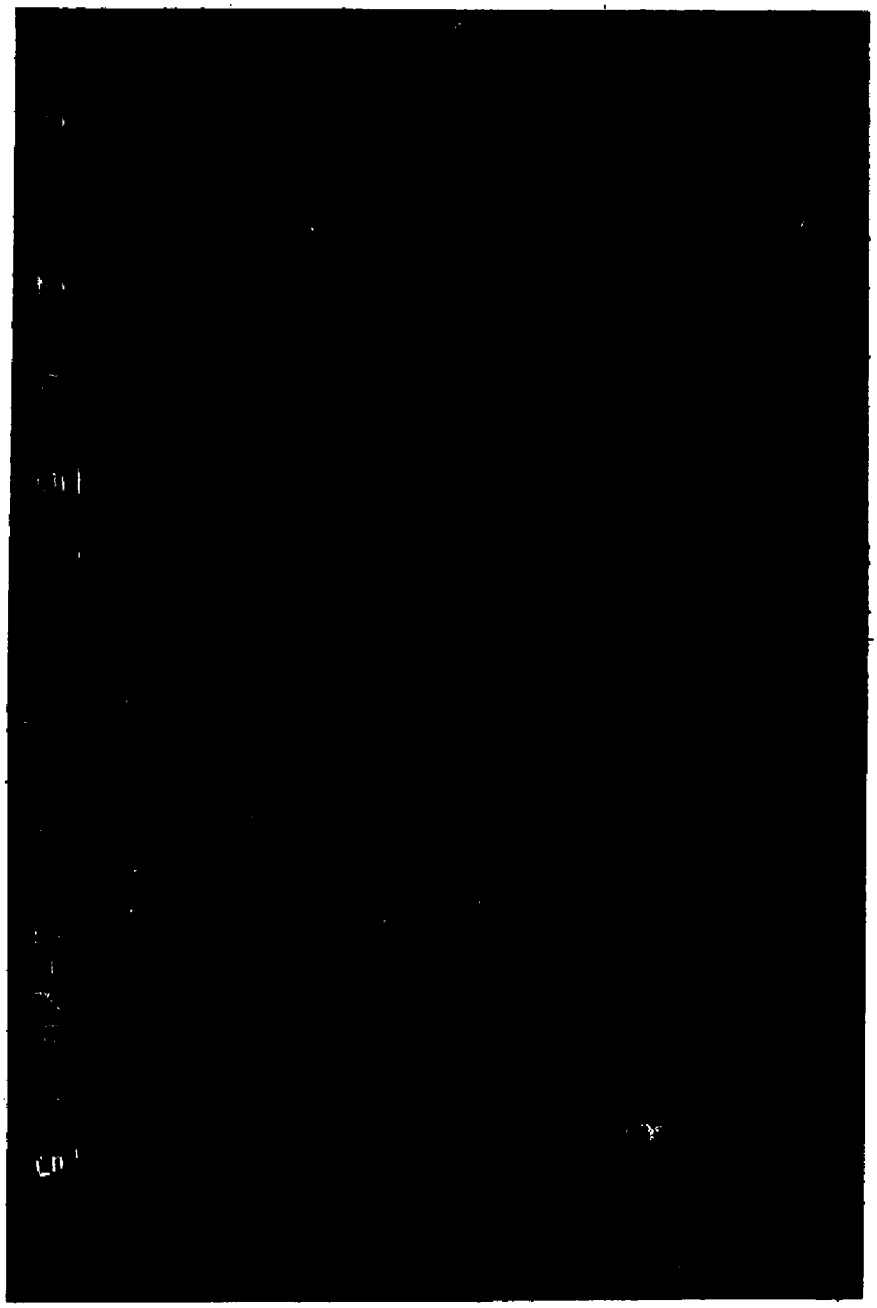
Table 6 - Effect of salt level, casing size, and endpoint temperature on smokehouse yields and Instron shear values.

Casing Size (cm)	Temperature (°C)	Salt (%) 1%	2%	3%
		Smokehouse Yield (%)		
9.6	67	66.34^{b}	71.39^{c}	84.06^{de}
9.6	74	61.96^{a}	67.63^{b}	80.28^{d}
13.6	67	78.10^{d}	81.02^{d}	88.58^{e}
13.6	74	73.95^{c}	77.05^{d}	87.30^{e}
		SE = 0.70		

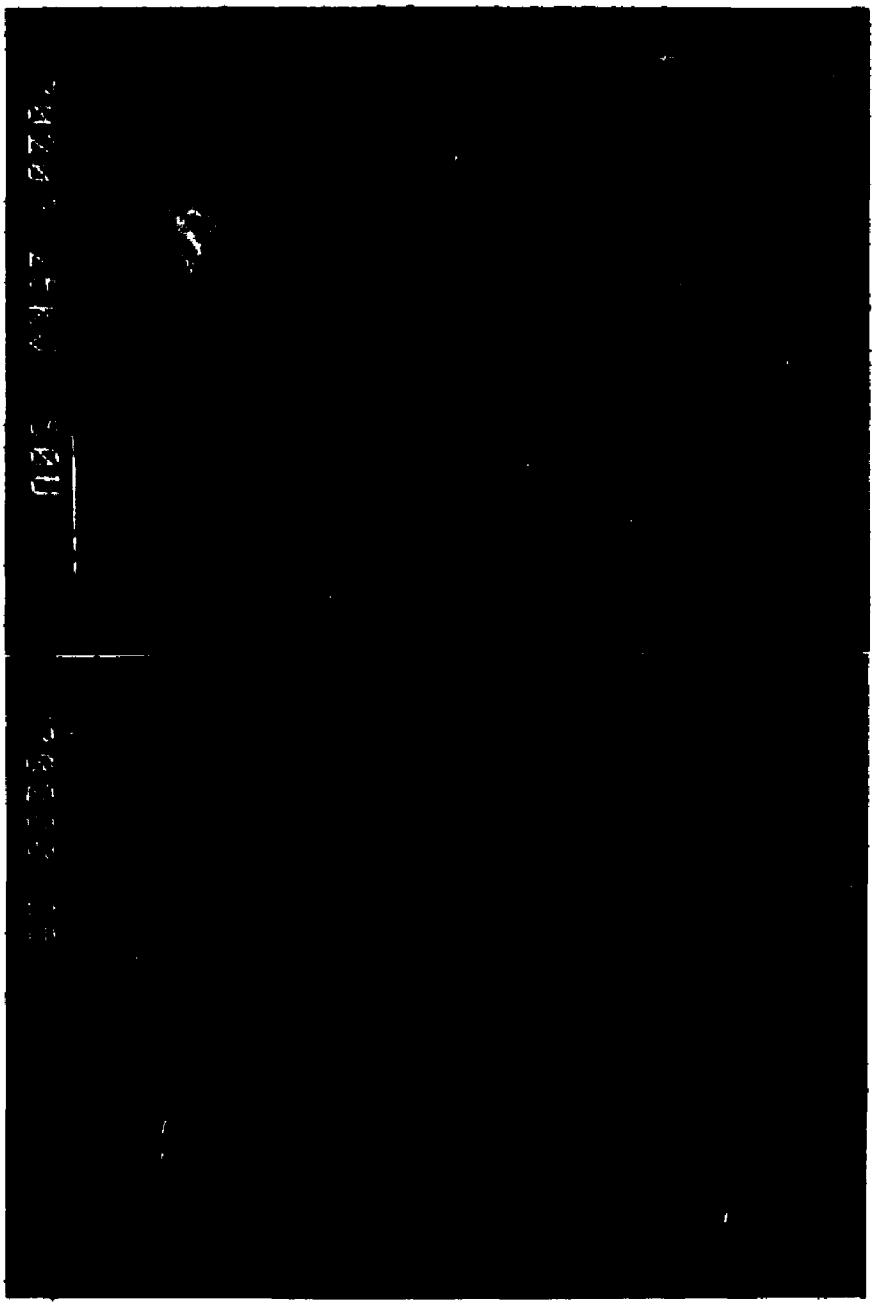
Casing Size (cm)	Temperature (°C)	Salt (%) 1%	2%	3%
		Instron shear value (kg/cm^2)		
9.6	67	9.22^{cde}	7.41^{bc}	4.09^{a}
9.6	74	10.41^{e}	8.71^{cd}	4.86^{a}
13.6	67	8.57^{cd}	6.92^{b}	4.06^{a}
13.6	74	9.03^{de}	7.47^{bc}	4.00^{a}
		SE = 0.45		

^{abcde}Least squares means with the same superscript in the same column or row are not different ($p>0.05$).

Micrograph 6 - SEM micrographs of bologna in 13.6 cm casing and cooked to 67°C endpoint temperature. A. 3% salt (500X). B. 3% salt (2,000X).

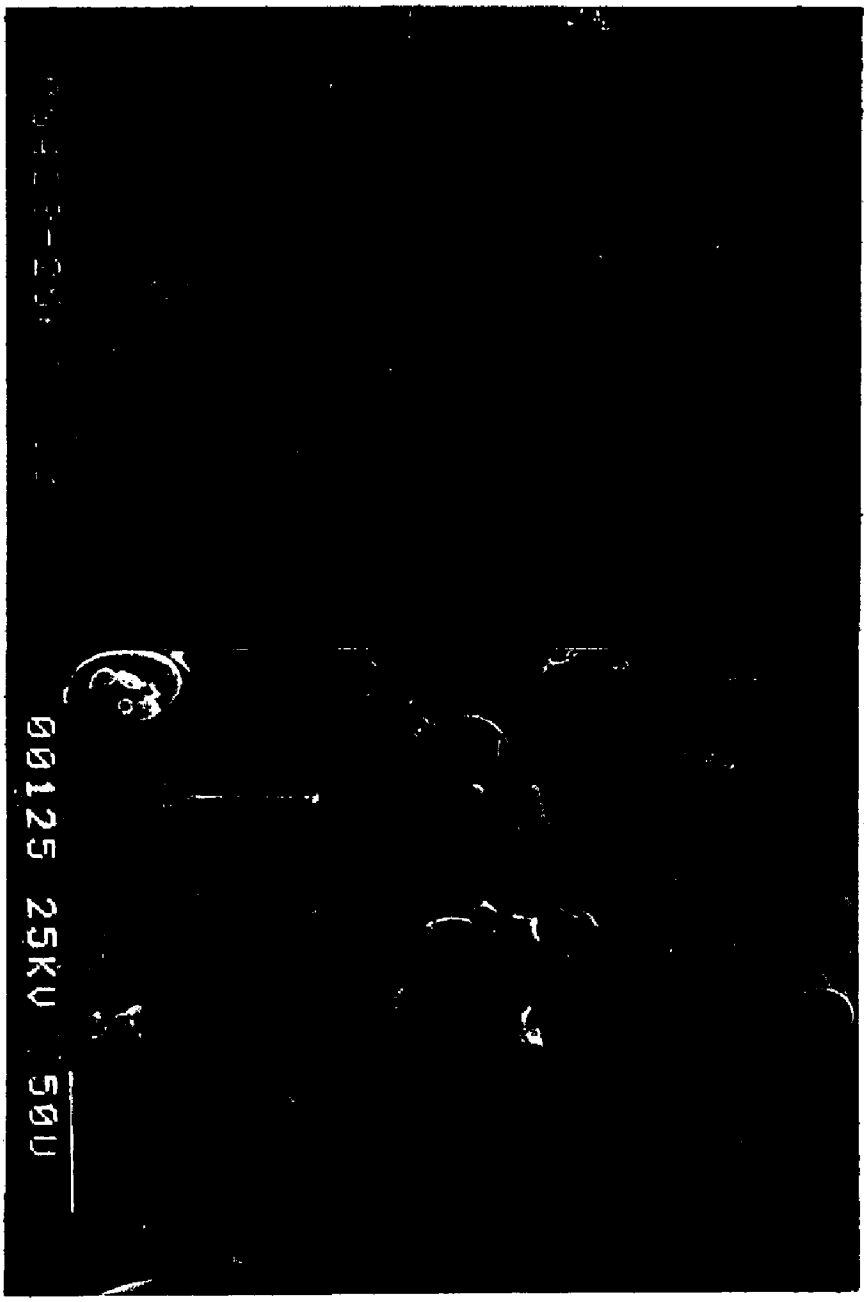


Micrograph 7 - SEM micrographs of bologna in 13.6 cm casing and cooked to 67°C endpoint temperature. A. 2% salt (500X). B. 2% salt (2,000X).

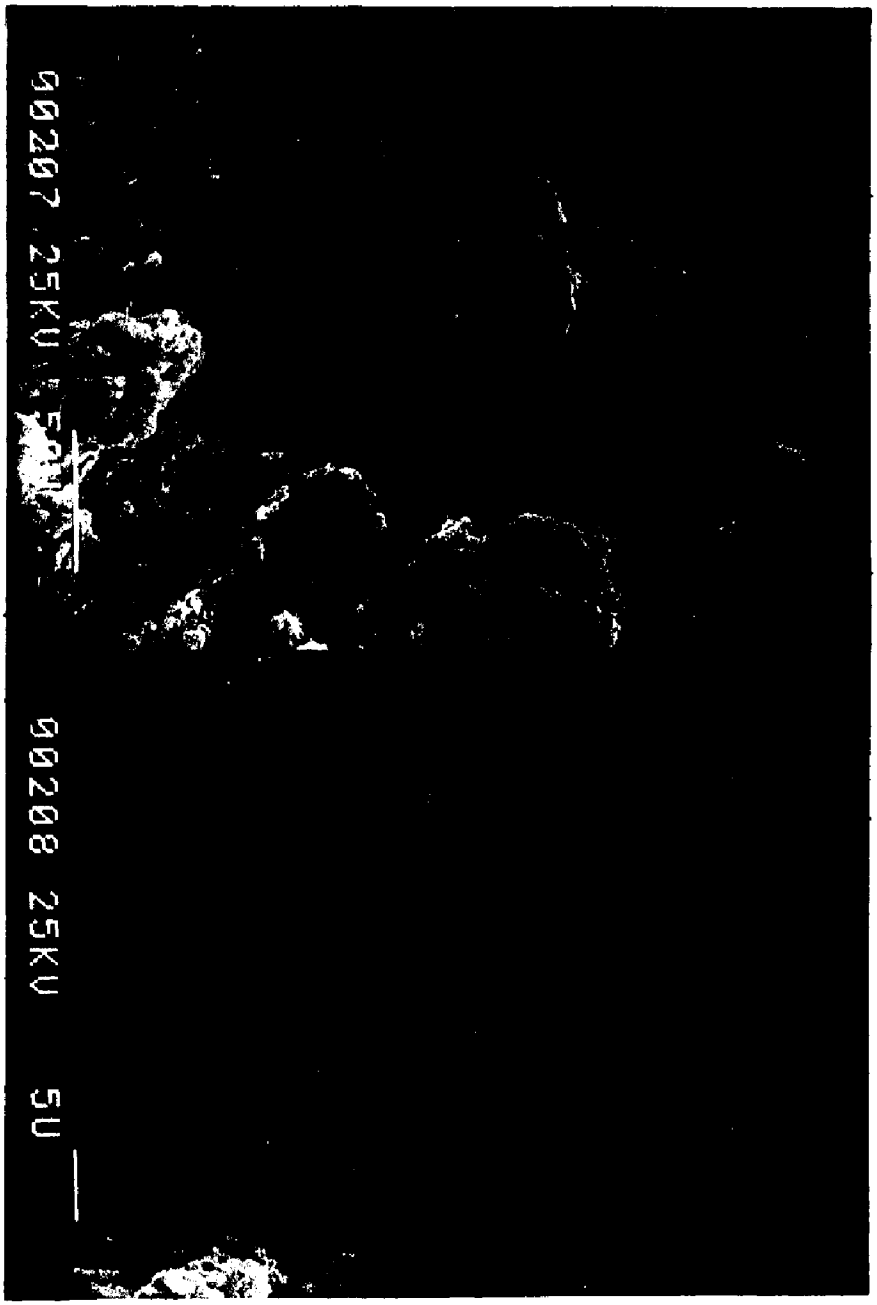


Micrograph 8 - SEM micrographs of bologna in 13.6 cm casing and cooked to 67°C endpoint temperature. A. 1% salt (500X). B. 1% salt (2,000X).

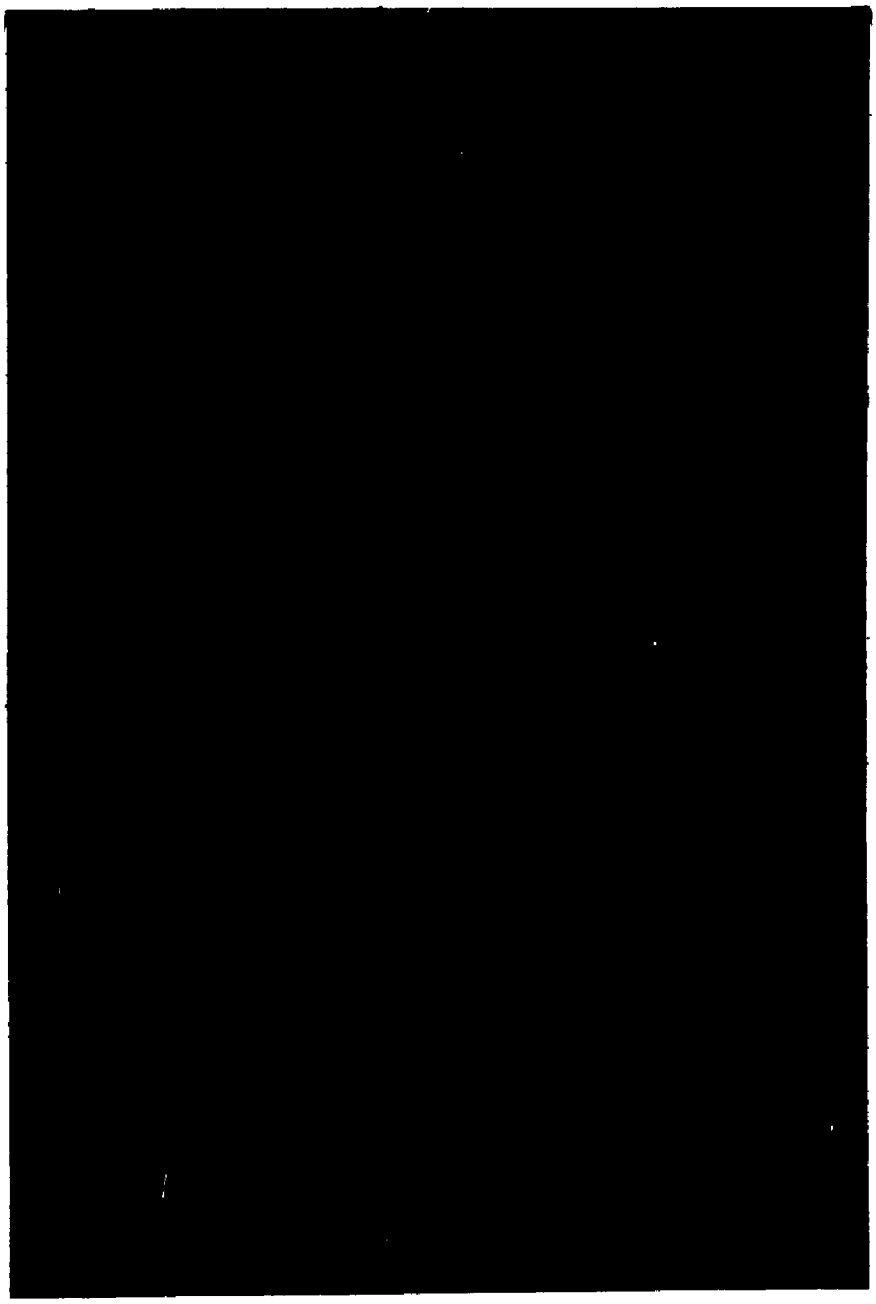
Micrograph 9 - Effect of casing size on microstructure of bologna at 67°C endpoint temperature and 3% salt. A. 13.6 cm (500X). B. 9.6 cm (500X).


00125 25KV 50U

Micrograph 10 - Effect of final endpoint temperature on microstructure of bologna in 9.6 cm casing with 3% salt. A. 67°C (500X). B. 67°C (2,000X).


00207 25KU
00208 25KU
5U

Micrograph 11 - Effect of final endpoint temperature on microstructure of bologna in 9.6 cm casing with 3% salt. A. 74°C (500X). B. 74°C (2,000X).



INFLUENCE OF SAMPLE PREPARATION UPON BOLOGNA MICROSTRUCTURE

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Beef bologna was manufactured with 10% ice and 3% salt, chopped to 18.3°C, stuffed into 13.6 cm casing and cooked to 67°C for evaluation of SEM microstructure. Three different sample preparation procedures (cryofracturing with no fixation, regular fixation, and Basgall's desiccation technique) were used to prepare samples for SEM study. The desiccator drying technique resulted in no structural differences compared to the chemical fixing and critical point drying techniques which have been widely used in most meat emulsion microstructure studies. The desiccator drying technique was relatively easy and rapid and capable of handling large numbers of samples at once as compared to the critical drying technique. The cryofracturing technique without fixation was not satisfactory in allowing identification of fat and protein components in bologna specimens.

INTRODUCTION

In recent years, electron microscopy has been shown to be a promising tool for evaluation of meat emulsion structures. Theno and Schmidt (1978) published SEM micrographs of meat emulsions which compared microstructural differences among three commercially produced frankfurters. Borchert et al. (1967) used both light and transmission electron microscopy to evaluate meat emulsions and found that fat globules as small as 0.1 um in diameter were surrounded by distinct membranes. Ray et al. (1981) examined the microstructure of liver sausage and reported that some of the fat globules were surrounded by a protein matrix in a lace-like network. Ray et al. (1979) used both light and scanning electron microscopy to identify fat and protein components in meat emulsions. The microstructure of frankfurters prepared with different endpoint chopping temperature was evaluated more recently by Jones and Mandigo (1982). Usually, preparation of meat emulsion samples for SEM requires fixation, dehydration and critical point drying. The method which most researchers have used is relatively slow for a large number of samples (Jones and Mandigo, 1982). Basgall et al. (1983) developed a faster procedure of sample preparation which used the usual initial cryofracturing and exposure of

surface components to the fixative solutions then employed a desiccator drying technique instead of a critical point drying technique. Cryofracturing of biological samples for SEM studies has been very useful in other food systems; however, limited usage of cryofracturing was found on emulsion-type sausage products. There was no direct comparison of different sample preparation techniques on emulsion-type sausage microstructure in the study of Basgall et al. (1983). The purpose of the present study was to compare the microstructure of bologna specimens prepared by cryofracturing with no fixation or regular fixation followed by either critical point or desiccation drying.

MATERIALS AND METHODS

Bologna was prepared with lean beef trimmings and pork backfat in batch sizes of ten kg. Unfrozen lean beef was ground through a 0.93-cm plate, then chopped in a Hobart silent cutter (model 1200) with 10% ice and 3% salt to 4.5 ^{o}C. Pork backfat (to comprise 25% of the meat block), spices and curing ingredients were added and chopping was continued to 18.3^{o}C. The meat batter was stuffed into 13.6-cm cellulose casings and cooked to a final endpoint temperature of 67^{o}C. Bolognas were chilled to 4^{o}C and stored in a 4^{o}C cooler prior to slicing into 10 mm thick slices. Bologna slices were randomly selected for each of the three specimen preparation treatments.

In treatment 1, a 5x5x10-mm section from a randomly selected slice of each bologna was cut with a razor blade, treated in liquid Freon (-110^{o}C) for 30 seconds and then submerged and held in liquid nitrogen (-196^{o}C) until impact fracturing to expose the intended viewing surface. The frozen specimens were lyophilized in a cold vacuum chamber overnight.

The second specimen preparation technique (treatment 2) was cryofractured followed by chemical fixation (Basgall et al., 1983). Sliced bologna samples were cryofractured as described previously. The frozen

samples were removed from the liquid nitrogen, and fixed in 3% glutaraldehyde fixative buffered with 20 mM 2 (N-Morpholino) ethane sulfonic acid (MES) for 18 hr at $4^{o}C$, and postfixed with 1% OsO_4 (osmium tetroxide) in distilled water for 30 minutes. The samples were then washed twice in 20 mM MES, pH 6.0, for 5 minutes. Samples were dehydrated in a graded ethanol series of 25, 50, 75, 90, 100, 100, and 100% (ethanol : water, v/v) for 10 minutes at $23^{o}C$. The samples were dried in a vacuum desiccator attached to a running water aspirator for 3 hours (Basgall et al., 1983).

The third preparation (treatment 3) utilized chemical fixation followed by critical point drying. A 5x5x10-mm section was cut with a razor blade from a randomly selected slice of each bologna. Each slice was then broken by hand to expose the intended viewing surface. Samples were fixed as in the procedure of Basgall et al. (1983) above. After dehydration in the graded ethanol series, samples were critical point dried with liquid CO_2 as the transitional medium.

All dried samples from the three fixation treatments were mounted on 13-mm aluminum stubs with double-stick Scotch^{R} cellophane tape. A thin (100 Å) Au/Pd coat was applied onto each specimen with a diode sputtering unit (Hummer 1). Micrographs at 500 X and 2,000 X were taken on a Hitachi S-500 scanning electron microscope at an accelerating voltage of 25 KV, and were subjectively evaluated for clarity, sharpness and contrast.

RESULTS AND DISCUSSION

Biological samples for SEM studies are fixed in glutaraldehyde to preserve proteins and postfixed with osmium tetroxide to stabilize fat (Hayat, 1976). Examination of the unfixed cryofractured surface of representative bologna samples revealed a noticeable distortion of fat globules (Micrograph 12A). Cryofracturing the relatively high-fat samples introduced ice crystal artifacts and the lyophilization of unfixed samples further distorted the reviewing surface. The protein matrix and fat globules were not properly protected by the cryofracturing technique but were disrupted by the ice crystal formation following the lyophilization process. Surface details of the bologna were not distinctive or clear because there were artifacts introduced during the freezing process. Clumped globule-type structures were observed; however, no distinguishing features between the protein matrix and fat globules as described by Ray et al. (1979) were visible. Micrograph 12B, the 2,000 X magnification of the protein matrix from the cryofractured unfixed sample, showed no distinguishing structural details of the fat and protein components.

Micrographs 13A (500 X) and 13B (2,000 X) show the exposed surface of bologna samples prepared with treatment

2. A homogeneous meat emulsion with evenly dispersed uniformly-sized fat globules was observed. The protein matrix and fat globules were well preserved by fixing in glutaraldehyde and osmium tetroxide. Glutaraldehyde is a non-coagulative fixative that forms molecular cross links and adds to structural stability (Hayat, 1976). Postfixation with osmium tetroxide has an additional advantage in that it imparts a modest conductive property to the fixed tissue (Hayat, 1976). Similar protein matrix and fat globule structures were observed by others (Theno and Schmidt, 1978; Ray et al., 1979; Jones and Mandigo, 1982). Many fat globules ranging from 3 um to 50 um in diameter can be seen scattered throughout the matrix. The protein matrix seems to be finely textured with encapsulated fat globules. Membrane-type structures or a relatively thick coat of protein formed threadlike structures on the surface of the encapsulated fat globules, much like the SEM micrographs of crude myosin and actomyosin gels prepared by Siegel and Schmidt (1979). The microstructure of the surface of bologna samples from treatment 3 is shown in Micrographs 14A (500 X) and 14B (2,000 X). The encapsulated fat globules are evenly distributed throughout the protein matrix. There were no major differences in the identification of microstructural components in these micrographs as compared to Micrographs 13A and 13B. Both treatment 2 and 3 would appear to preserve structural details in cooked meat samples.

In summary, the cryofracturing, chemical fixation and desiccator drying technique developed by Basgall et al. (1983) provided no structural differences compared to the chemical fixing and critical point drying technique which has been widely used in most meat emulsion microstructure studies. However, the desiccator drying technique is relatively easy and rapid and capable of handling large numbers of samples simultaneously as compared to the critical point drying technique. The cryofracturing technique with no fixation step was not satisfactory in allowing clear or easy identification of fat and protein components in bologna specimens.

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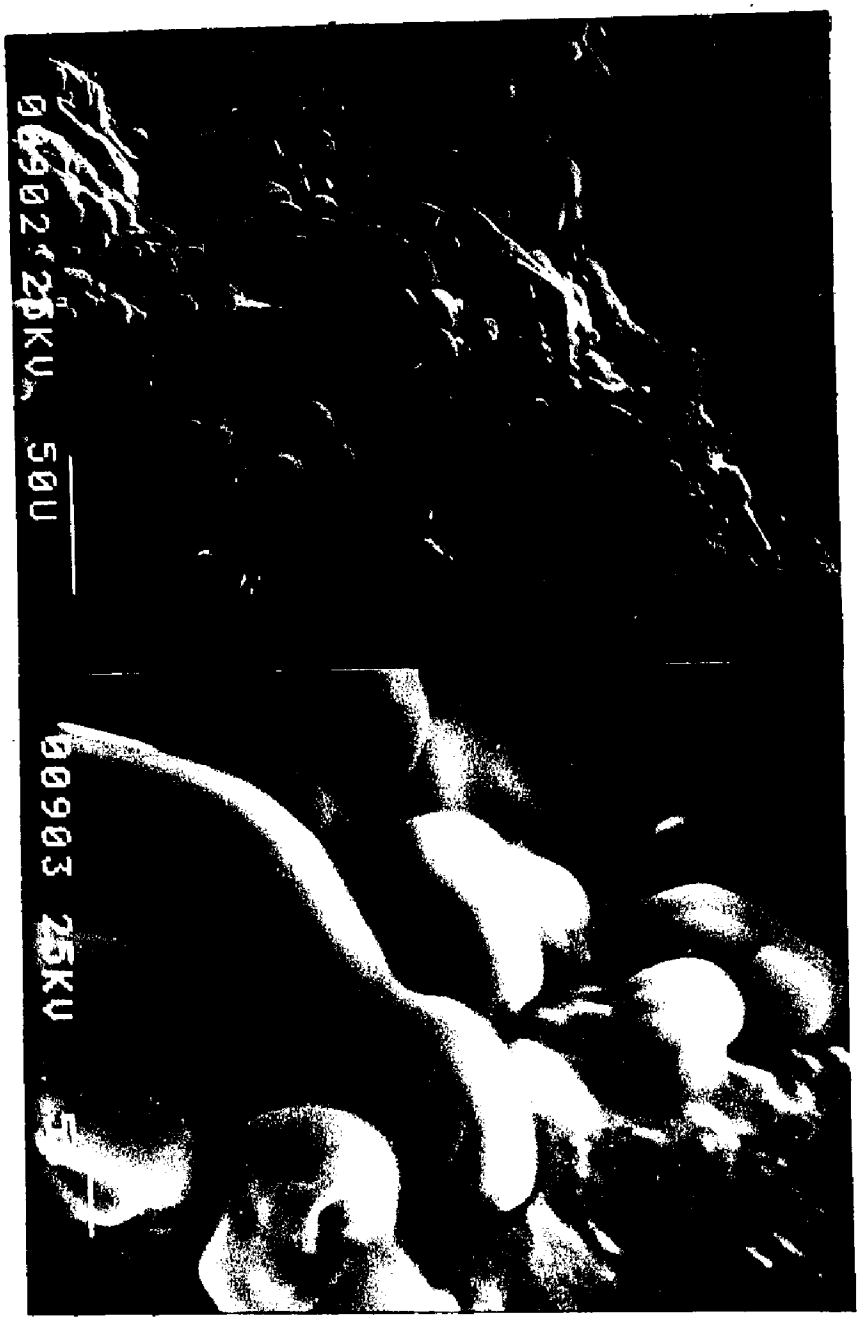
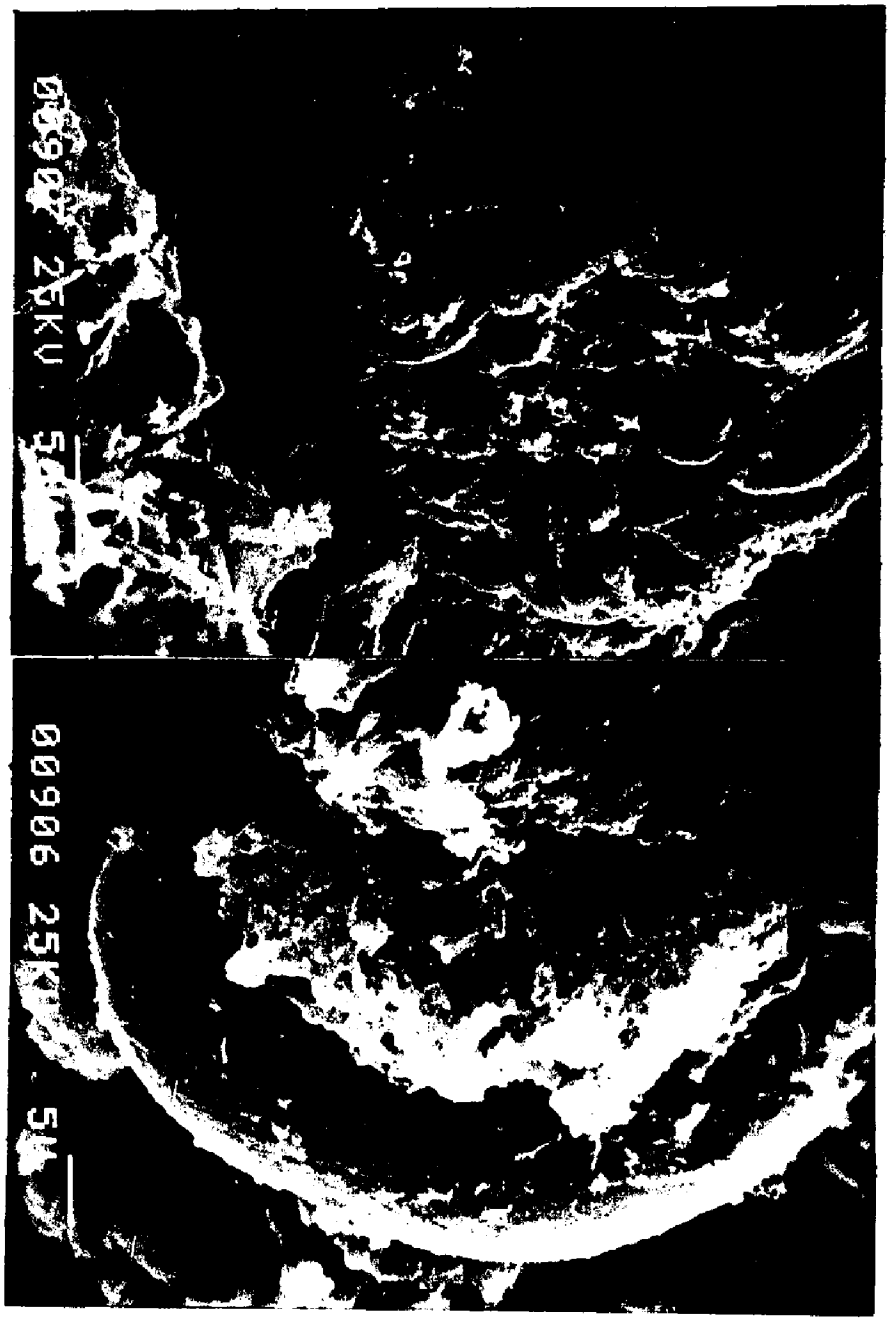
Micrograph 12 - Micrographs of the cryofractured unfixed surface of bologna samples. A. 500X B. Enlargement of indicated square area (2,000X).


00902 25KV 50U
00903 25KV 5

Micrograph 13 - Micrographs of the fixed and desiccator dried bologna samples. A. 500X B. Enlargement of indicated square area (2,000X).


5M
25KU 900906
5M
37GHZ 24KU 900906

Micrograph 14 - Micrographs of the fixed and critical point dried bologna samples. A. 500X B. Enlargement of indicated square area (2,000X).


1μm
21600

EFFECTS OF PORK FAT SOURCE AND TYPE OF PACKAGING UPON THE OXIDATIVE STABILITY OF FRANKFURTERS

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EFFECTS OF PORK FAT SOURCE AND TYPE OF PACKAGING UPON THE OXIDATIVE STABILITY OF FRANKFURTERS. Y. C. Wu, K. W. McMillin, J. S. Godber, and T. D. Bidner. Department of Animal Science, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

The effects of vacuum and non-vacuum packaging on frankfurters manufactured with three sources of pork fat were monitored during a nine-week storage period using peroxide values and TBA numbers as indicators of oxidative stability. There were no significant compositional differences in frankfurters prepared with prerigor fat, postrigor fat or lard. Analysis of peroxide values showed significant two-way interactions between the fat source and packaging type and the fat source and time of storage. The peroxide values were lower ($p<0.05$) for vacuum packaged frankfurters made from lard than for those which were non-vacuum packaged. Peroxide values of frankfurters from all fat groups decreased ($p<0.05$) as time of storage increased. Vacuum packaging decreased ($p<0.05$) the TBA numbers in frankfurters prepared with postrigor fat and lard compared to nonvacuum packaging. A decrease ($p<0.05$) in TBA numbers occurred at 6 and 9 weeks for prerigor fat frankfurter groups while TBA numbers of frankfurters with postrigor fat increased ($p<0.05$) after the first week of storage. TBA numbers of frankfurters manufactured with lard increased at

six weeks of storage but declined to initial levels at nine weeks of 4^{o}C storage.

INTRODUCTION

The processing of pork products immediately after the slaughter of the animal has been described as "hot processing" or "accelerated processing". The economic potential of accelerated pork processing has been recognized (Henrickson, 1979; Mandigo, 1968) as a means to save energy by chilling only the edible portion of the carcass, reduce the requirements for refrigeration facilities, reduce transportation cost, and reduce labor requirements (Ray et al., 1980). There is an advantage to preblending hot boned meat for emulsion-type products because an increased emulsion capacity can be utilized (Acton and Saffle, 1966). Only a few meat packing plants have adapted hot processing because it is perceived that prerigor boning is difficult due to the rubbery texture and because there may be substantial economic investments in altering product flow or processing techniques in existing facilities. Tadic (1966) compared emulsions of animal fat tissues and rendered fats derived from the same location of the animal and reported an increased stability of emulsions produced with fat tissue, but a finer fat particle size in emulsions with rendered fats.

The oxidation of lipids has been identified as a major cause of quality deterioration in stored meat and meat

products (Love, 1983). Wilson et al. (1976) found that phospholipids were major contributors to rancidity in beef. In addition to affecting palatability factors, such as meat flavor and color, lipid oxidation produces compounds which have adverse biological effects. Drerup et al. (1981) demonstrated that prerigor grinding and salting reduced the rate of autoxidation in pork sausage during storage at 0°C. Judge and Aberle (1980) reported that prerigor ground pork muscle was less susceptible to oxidative stability development than postrigor ground pork. Little reference information, however, is available on the oxidative stability of prerigor fat blended with postrigor muscle tissue. In this experiment, the interaction of three fat treatments and vacuum packaging on peroxide and TBA values in frankfurters was investigated. A non-vacuum treatment was used to promote conditions for oxidation so that the effect of fat source on oxidative stability would be enhanced.

MATERIALS AND METHODS

The subcutaneous fat areas from eight pork carcasses of pigs slaughtered in the LSU meat laboratory were randomly assigned to one of three treatments: prerigor (warm), postrigor (chilled) fat and chilled lard. The carcass fat areas assigned to the warm fat treatment were removed no later than one hour post-mortem and immediately incorporated into the manufacture of frankfurters. The postrigor (chilled) fat was removed 18 hours post-mortem and held at 2°C for 3 days before incorporation into frankfurter batters. A portion of the chilled fat was processed into lard by steam rendering (95°C), straining and re-chilling to 4°C before inclusion in the emulsion batters. The three types of pork fat were the only sources of fat in the frankfurter batters.

Postrigor beef which had been trimmed of all visible fat was placed in a 3-bladed Manurhin bowl cutter with 3% salt and 10% ice. When chopping temperatures had reached 4°C, the designated treatment of pork fat was added to comprise 26% of the meat block and chopping continued to a temperature of 18°C. The final emulsion batter was stuffed into 22 mm cellulose casings and thermally processed in a smokehouse (20 minutes at 56°C and 36.5% relative humidity (RH), 30 minutes at 65.5°C at 56% RH and 93°C and 60% RH to

67°C internal temperature). After chilling to 4°C and peeling, frankfurters were randomly divided into two groups for vacuum or non-vacuum packaging. Frankfurters for vacuum packaging were placed into saran coated polyethylene pouches where pressure was decreased to 80 mm Hg before heat sealing. Frankfurters that were not vacuum packaged were placed into saran coated polyethylene pouches.

The vacuum and non-vacuum packages of frankfurters were stored at 2°C for 63 days. Moisture, crude fat and Kjeldahl protein were determined on frankfurters by AOAC (1980) procedures. Oxidative stability was determined by measuring TBA numbers (Tarladgis et al., 1960) and peroxide values (Ockerman, 1980) on days 1, 7, 21, 42 and 63 of the storage period. The frankfurter samples were blended with cold deionized water and duplicate aliquots of homogenate were used for determination of TBA number (mg malonaldehyde/kg sample). Sulfanilamide was added at the blending stage to prevent the reaction of nitrite and malonaldehyde during the distillation step. Lipids were extracted from the frankfurter samples by the method of Bligh and Dyer (1959), and were used for the determination of peroxide value expressed as milliequivalents of peroxide per kg oil.

A split plot design with two replications was analyzed using fat sources as main plot. Vacuum condition and storage period were considered as treatments nested within fat sources (Snedecor and Cochran, 1980). The general

linear models procedure of SAS (1979) was used for peroxide value and TBA number analyses. The statistical model was:

$$Y = u + R + F + Ea + V + T + (F \times V) + (F \times T) + (V \times T) + (F \times V \times T) + Eb + Es;$$

where R = effect due to replications,

F = effect due to fat sources,

Ea = (RxF) error term for main plot,

V = effect due to vacuum conditions,

T = effect due to storage periods,

F X V = interaction between F and V,

F X T = interaction between F and T,

V X T = interaction between V and T,

F X V X T = interaction among F, V, and T,

Eb = RxTxV(F) error term for sub plot treatments,

and Es = sampling error used to test Eb.

Least squares means were employed for statistical separation of individual treatment means.

RESULTS AND DISCUSSION

The proximate analyses of the frankfurters on day 1 of the study are presented in Table 7. The moisture, protein and crude fat contents of frankfurters were not different among the three fat treatment groups. The sums of squares for peroxide value and TBA number are shown in Appendix Table 9. Main effect of fat source was significant ($p<0.01$) for peroxide value and for TBA number ($p<0.01$). Storage time caused highly significant ($p<0.01$) differences in peroxide value and TBA number. Peroxide values and TBA numbers were influenced ($p<0.05$) by two-way interactions ($p<0.05$) of fat source and packaging method, and fat source and time of storage period. The two-way interaction of packaging method and time of storage for TBA number was also significant ($p<0.05$).

The influences of fat source and packaging treatment on peroxide values and TBA numbers are shown in Table 8. Peroxide value measures the primary oxidation products and TBA number measures secondary oxidation products of fat oxidation (Ockerman, 1980). Peroxide value is a useful indicator of the onset of oxidation and TBA number reflects the secondary reactions of oxidation. Vacuum packaging did not greatly affect peroxide values of frankfurters produced from pre- and postrigor fat. The vacuum packaged

frankfurters made with lard had lower ($p<0.05$) peroxide values than frankfurters made with lard that were not vacuum packaged. The chilled or postrigor fat resulted in frankfurters with the lowest ($p<0.05$) peroxide values compared to frankfurters from the prerigor and lard groups. Grinding or chopping of warm fat might cause more fat cell disruption and phospholipid exposure than grinding of chilled fat and this might be responsible for the relatively high initial peroxide values of the prerigor fat group. TBA numbers of the frankfurters (Table 8) made with prerigor fat or lard were not affected by packaging treatment. In the frankfurters produced with chilled postrigor fat, vacuum packaging significantly ($P<0.05$) decreased TBA number compared to non-vacuum packaging.

The influence of storage time on fat oxidation is shown in Figure 1. The peroxide values of all frankfurters decreased ($p<0.05$) with increased time of storage in the 9-week period. Other researchers have shown that peroxide values of meat also decrease with storage time (Ockerman, 1980; Judge et al., 1981). The lard-prepared frankfurters had greater peroxide values than the frankfurters prepared from prerigor and postrigor fat after the first week of storage.

The TBA numbers of the lard and prerigor fat frankfurters did not change until three weeks of storage at $2^{\circ}C$ (Figure 2). At six and nine weeks, the frankfurters from prerigor fat had lower ($p<0.05$) TBA values than during

the previous three weeks. After the first week, TBA numbers of frankfurters prepared with postrigor fat increased ($p<0.05$) through the sixth week of storage but declined when frankfurters were tested at nine weeks. The frankfurters from lard showed elevated ($p<0.05$) TBA numbers at six and nine weeks compared to 1, 7 and 21 day storage periods. At three and six weeks of storage, TBA numbers of frankfurters from the postrigor fat were higher ($p<0.05$) than for frankfurters from lard. This might have been caused by removal of phospholipids during the rendering process used in the production of the lard. The increases in TBA number with storage time for postrigor and lard frankfurters would indicate loss of reducing capacity to maintain myoglobin in a reduced form. Verma (1985) reported that denatured myoglobin and denatured metmyoglobin were catalysts of lipid oxidation in emulsions. In the present study, TBA numbers of frankfurters were in the threshold range (Tarladgis et al., 1960; Greene and Cumuze, 1982) of indicating detectable oxidized flavors.

The influence of packaging and time of storage on TBA number is shown in Figure 3. There were no significant differences in TBA numbers of vacuum packaged frankfurters among storage periods; however, TBA numbers increased ($p<0.05$) at six weeks of storage and decreased ($p<0.05$) at nine weeks of storage in non-vacuum packaged frankfurters.

Frankfurters manufactured with warm (prerigor) fat showed lower TBA numbers compared to frankfurters with

chilled (postrigor) fat and lard. Vacuum packaging increased the oxidative stability in frankfurters of all fat-treatment groups. Prerigor fat appeared to be less susceptible to oxidative rancidity development than postrigor fat, but the chemical differences between pre- and postrigor fat are not clear. This study indicated that the use of prerigor fat in frankfurter formulations might increase oxidative stability during storage, but further research in this area would elucidate the effects of fat types or temperature upon other properties of processed meat products.

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Table 7 - Proximate composition of finished frankfurters manufactured with different fat sources.

Fat Source	n	Moisture %	Protein %	Crude Fat %
Prerigor	10	57.98	13.07	24.89
Postrigor	10	58.18	12.98	25.10
Lard	10	57.89	13.15	24.78
	SE	.96 NS	.24 NS	.71 NS

NS Means in the same column are not significantly different (P>0.05).

Table 8 - Effect of fat source and packaging on peroxide values and TBA numbers.

Treatments Fat	Packaging	n	Peroxide Value Initial	Peroxide Value Overall	TBA Number Initial	TBA Number Overall
Prerigor	Vacuum	10	2.47^{c}	1.49^{bc}	0.93^{b}	0.83^{a}
	Non-vacuum	10	2.52^{c}	1.26^{b}	0.87^{b}	0.74^{a}
Postrigor	Vacuum	10	1.92^{b}	0.94^{a}	0.72^{b}	0.80^{a}
	Non-vacuum	10	1.52^{a}	0.88^{a}	0.51^{a}	1.00^{b}
Lard	Vacuum	10	2.01^{b}	1.30^{b}	0.87^{b}	0.86^{a}
	Non-vacuum	10	1.94^{b}	1.61^{c}	0.81^{b}	0.91^{ab}
		SE		0.10		0.04

abc Least square means in the same column with the same superscript are not significantly different (P>0.05).

Figure 1. Effect of different fats and storage periods on the peroxide values

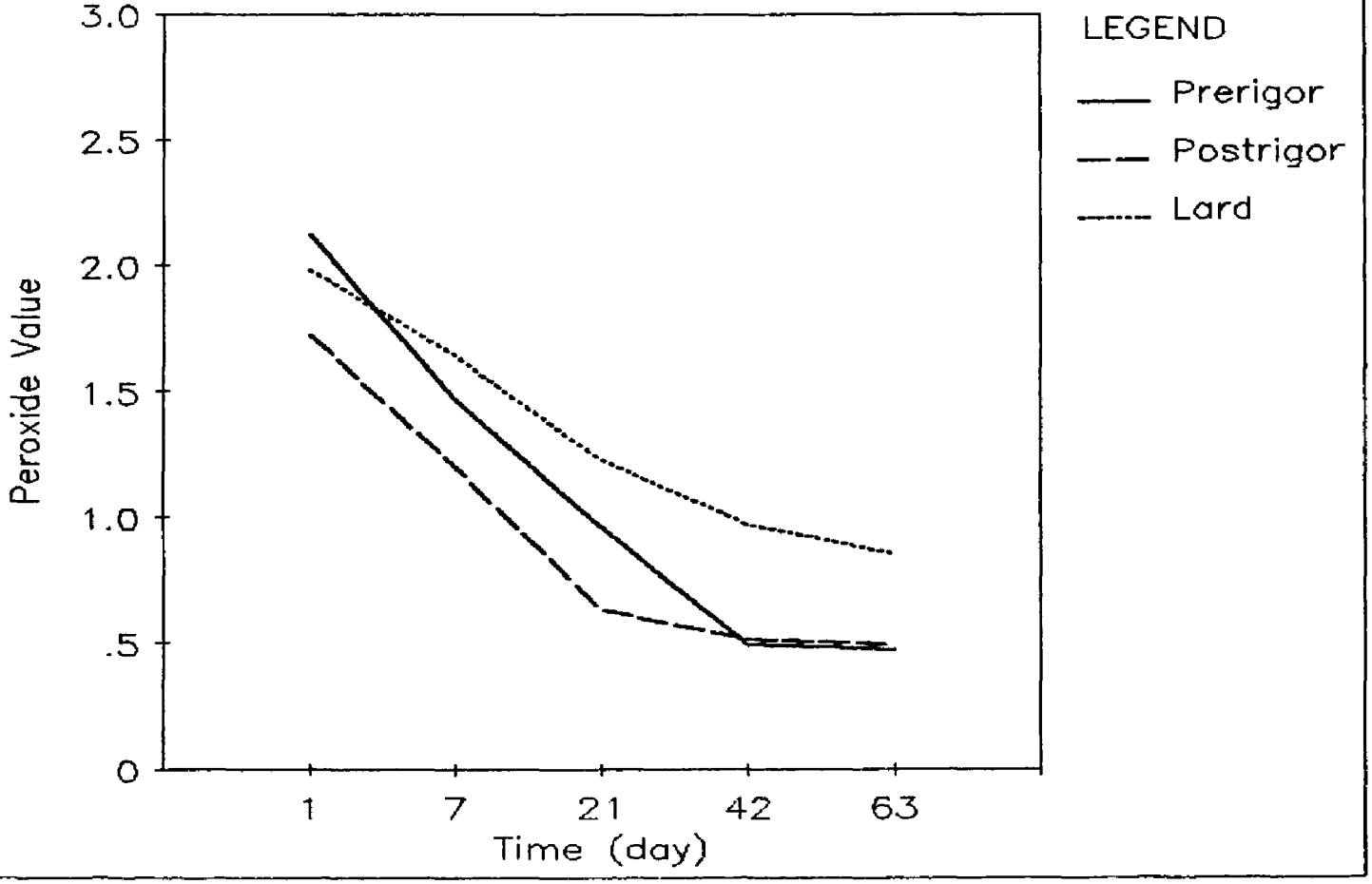


Figure 2. Effect of different fats and storage periods on the TBA numbers

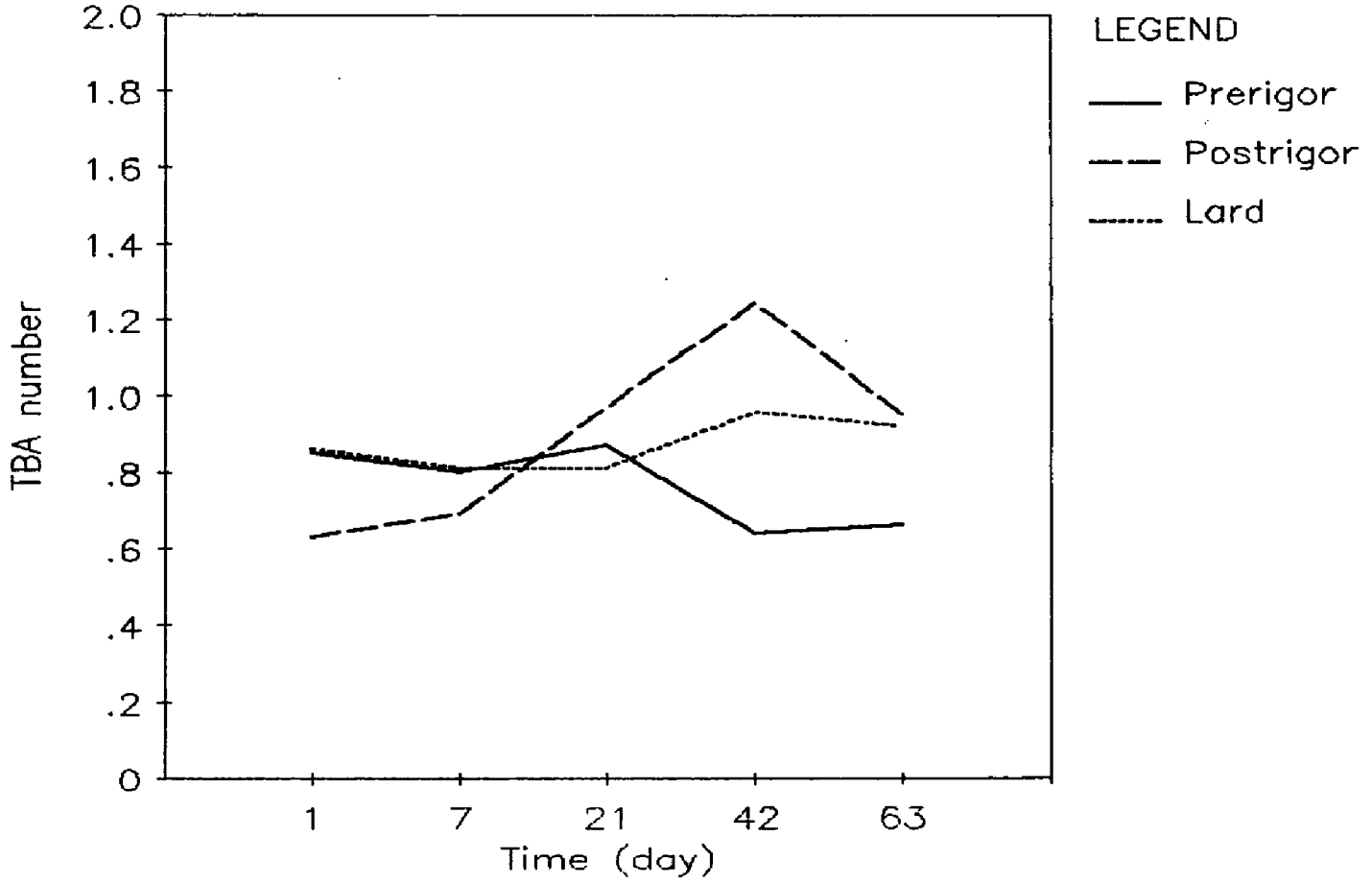
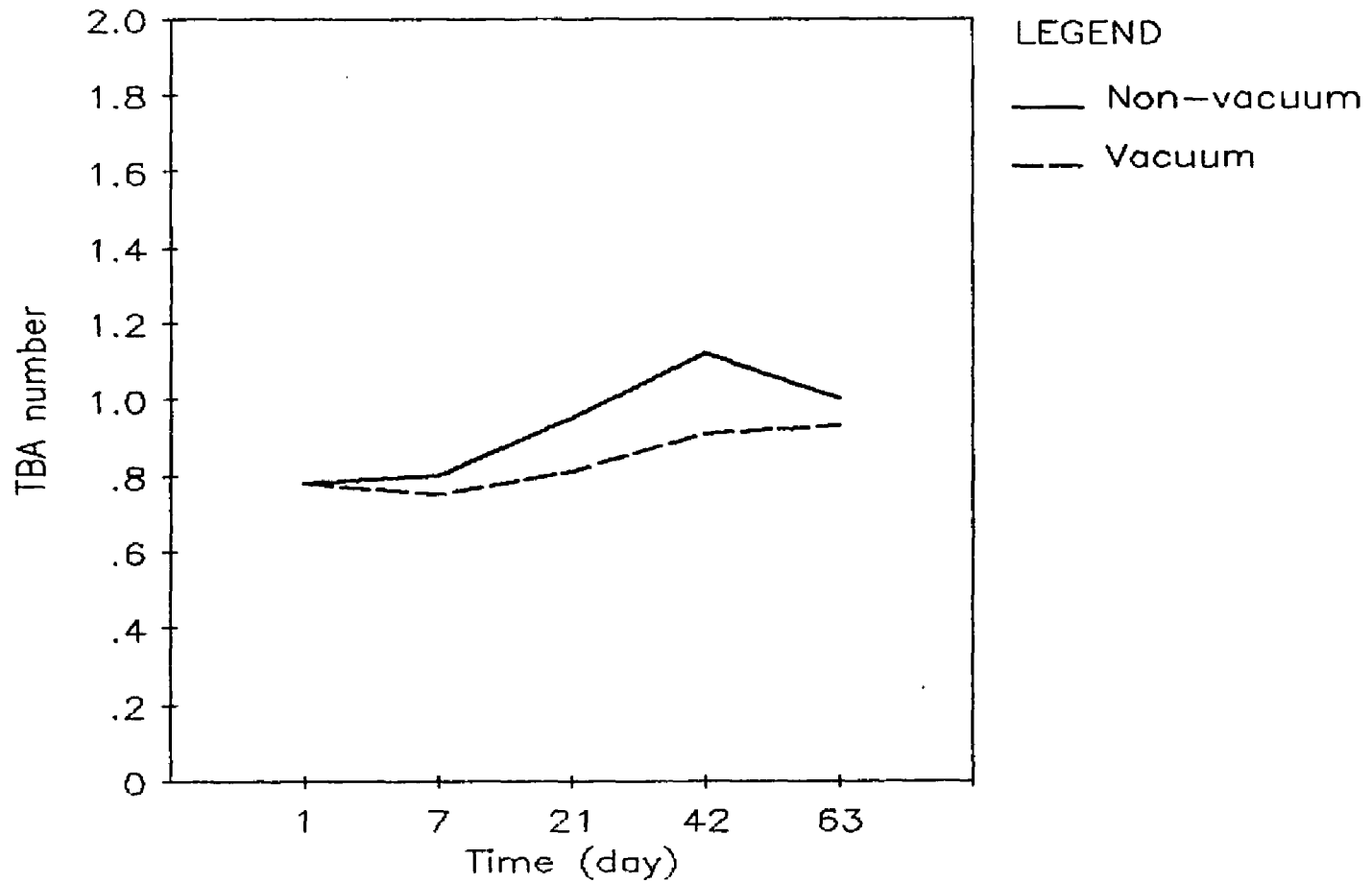


Figure 3. Effect of packaging and storage periods on the TBA numbers



SUMMARY

The influences of different processing technologies on the properties and microstructures of emulsion-type sausage products were investigated.

In experiments one and two, beef bologna was manufactured with different salt levels, chopped to 10, 15.5 and 21°C, stuffed into 9.6 and 13.6 cm casings and cooked to 67 or 74°C for evaluation of water binding capacity and emulsion stability of raw emulsions and smokehouse yield, Instron shear value and scanning electron microscopy (SEM) microstructure. Treatments with lower salt levels had higher total oil and water losses and lower water binding capacity. Emulsion stability tended to increase as chopping temperature increased in the 3% salt group. The thermal processing served to heat coagulate the solubilized protein that coated the fat globules. Samples with 1 or 2% salt had higher smokehouse yield with 67°C endpoint cooking temperature than 74°C endpoint cooking temperature. Less heat denaturation occurred in 13.6 cm casings than for bologna in 9.6 cm casings. SEM micrographs showed that the 3% salt treatment had a thicker protein coating and more stable emulsion as compared to 1 or 2% salt treatments, which had a thinner protein coating and less stable emulsion. The use of salt below 2% in emulsion-type sausage

products resulted in a decrease in smokehouse yield, lower water binding capacity and emulsion stability. Reduction of sodium chloride to 1.5%, or 1% resulted in reduced emulsion stability and increased smokehouse losses. With reduced salt levels, meat processors need to consider alternative processing techniques such as preblending of prerigor meats, partial replacement of NaCl with KCl or other chloride salts and addition of phosphate salts or NaOH, if conventional bologna characteristics are the desired result. SEM micrographs showed a multiphasic system. Fat was dispersed in a complex matrix of soluble proteins, fragments of muscle fibers and connective tissue. Protein-to-protein interactions are as important as protein-to-fat interactions. The interactions of purified protein (myosin) and purified fat (lard) may be a research interest in the future.

Since artifacts in bologna microstructure may be introduced during sample preparation, in experiment three, bologna specimens prepared from cryofracturing with different fixation were used to compare the differences in microstructures. Desiccator drying technique were compared to the chemical fixing and critical point drying technique that has been widely used in most meat emulsion microstructure studies. The cryofracturing technique with no fixation step was not satisfactory in allowing identification of fat and protein components in bologna specimens.

Hot-boning offers distinct advantages in labor, space and energy conservation. Prerigor meats have greater emulsifying capacity, water binding capacity and usually lower microbial counts. Preblending of prerigor meats is important in low-salt sausage production. In experiment four, the effect of different fat sources, packaging conditions and storage periods on the development of oxidative rancidity in frankfurters was studied. Lower TBA number was observed under vacuum packaging for all frankfurter samples. The peroxide values were lower for vacuum packaging than for non-vacuum packaging. Frankfurters manufactured with prerigor (warm) fat showed lower TBA number as compared to frankfurters manufactured with postrigor (chilled) fat and lard. Further research is recommended in the area of differences in types of fat used in processing of hot-boned meat products to further characterize influences on emulsification and bind properties among different protein and fat sources.

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APPENDICES

Appendix Method 1

Moisture Determination

1. Dry, clean, aluminum pans for at least two hours in the drying oven at 100°C. With tongs, place pans in a desiccator to cool (1 hour). Weigh to 4 decimal places in grams.

2. Rapidly transfer approximately 10 grams of sample to aluminum pan, spread sample over bottom of pan, and weigh pan and sample.

3. Place pan and sample in a drying oven set at 100°C for 18 hours. Remove to desiccator, cool (1 hour), and weigh pan and sample.

4. To obtain percent moisture:

$$\% \text{ Moisture} = \frac{\text{Weight loss (g)}}{\text{Sample weight (g)}} \times 100$$

5. Place pans back in a desiccator and run fat on these dried samples.

Appendix Method 2

Crude Fat Determination

1. Obtain samples from moisture determination (oven dry).
2. Dry in oven (2 hours at 100°C) soxhlet thimbles, cool in desiccator (1 hour) and weigh.
3. Transfer with the aid of ether, dried sample from aluminum pan to thimble.
4. Place thimble in soxhlet extractor. Add ether to cold extractor.
5. Pass cold water through condenser and check flow. Plug in extractor, set reostat at 25. Extract sample for 8 hours.
6. Let extractor cool before removing thimble. Remove thimble and let ether evaporate in hood for 2 hours. Then place thimble in drying oven for 2 hours, cool in a desiccator and weigh.
7. % Crude fat (wet weight basis) =

$$\frac{\text{Weight loss during ether extraction (g)}}{\text{Original wet sample weight (g)}} \times 100$$

Appendix Method 3

Kjeldahl Protein Determination

1. Weigh a piece of dry filter paper. Add approximately 2.5 g of sample and re-weigh paper and sample to 4 decimal places.

2. Place sample and paper in a clean 800 ml Kjeldahl flask.

3. Add:
   a. 1 g of the catalyst mixture (10 parts of K_2SO_4 to 1 part $CuSO_4$).
   b. 3 glass beads.
   c. 25 ml of concentrated H_2SO_4.

4. Start fan and burners on digestion rack.

5. Place flask on digestion rack until solution is a clear blue-green; then, let digest for an additional 30 minutes.

6. Cool flask for 30 minutes.

7. To cool Kjeldahl flask add 400 ml of distilled water. Hold the flask in an inclined position while adding water.

8. Add 50 ml of 4% H_3BO_4 containing indicator to an Erlenmeyer flask. Place this flask under the condenser with glass tube immersed in the liquid.

9. Add two pieces of mossy zinc to Kjeldahl flask.

10. Carefully layer 75 ml of saturated NaOH in the flask. Hold the flask in an inclined position and let the NaOH flow under the solution in the flask and not mixing with it. Connect the flask and shake vigorously until it is a uniform color. After solution is well mixed, distill over 200 ml (250 ml total volume). Wash off tube in Erlenmeyer flask with distilled water.

11. Remove Erlenmeyer flask and replace with 400 ml of distilled water. Turn off heat and allow water to be drawn back through the distillation apparatus.

12. Titrate the boric acid solution with .0726N HCl solution to a steel gray or nearly colorless endpoint.

$$\% \text{ Protein} = \frac{(\text{ml HCl of sample} - \text{ml HCl of blank}) \times 1.4 \times 6.25 \times (\text{Normality of HCl})}{\text{Weight of sample (g)}}$$

Appendix Method 4

Ash Determination

1. Dry a porcelain evaporating dish overnight at 525°C in an ash oven. Cool in a desiccator (2 hours) and weigh to 4 decimal places in grams.
2. Add approximately 5 grams of meat to the evaporating dish and re-weigh.
3. Dry 18 hours in drying oven at 100°C.
4. Heat on hot plate in exhaust hood until completely charred and smoking stops.
5. Place in ash oven furnace for 18 hours at 525°C.
6. Cool in desiccator (2 hours) and weigh.
7. $$\% \text{ Ash} = \frac{\text{Weight of residue}}{\text{Weight of wet sample (g)}} \times 100$$

Appendix Method 5

Method for Water Binding Capacity

1. Remove a sheet of 9 cm Whatman # 1 filter paper from a desiccator containing saturated solution of KCl. Tare the paper.

2. Add 500 mg of sample to the paper.

3. Place filter paper containing meat sample between two plexiglass sheets. Press with the Carver Lab Press at 3,000 psi for 3 minutes.

4. Trace inner and outer surface of pressed meat and juice on acetate paper.

5. Measure inner and outer area with a polar planimeter.

6. Run % moisture on meat sample.

7. $$\% \text{ Free water} = \frac{(\text{total surface area} - \text{meat film area})(61.10)}{\text{total moisture (mg) of meat sample}} \times 100$$

8. % Bound water = 100 - % free water.

Appendix Method 6

Method of Emulsion Stability

1. Place a fitted glass disc into a Wierbicki tube, identify it with a wax pencil and record the weight of the tube.

2. To sample the emulsion mixture, use the open end syringe to slowly draw the emulsion into the body of the syringe. Be careful to avoid air bubbles in the syringe.

3. Fill the Wierbicki tube with about 25 grams of emulsion by resting the filled syringe on the glass disc, and simultaneously pushing the plunger and retracting the syringe. Keep some pressure on the emulsion so that it fills the tube without air pockets, but be careful not to force it past the glass disc.

4. Reweigh the filled tube to get actual sample weight.

5. Cook the samples in 160^{o}F water bath for 30 minutes. Remove and allow to cool for 2-3 minutes.

6. Centrifuge at low speed for 5 minutes.

7. Remove the tubes and read the amounts of separated fat (top layer) and separated water (bottom layer).

8.
$$\% \text{ water separation} = \frac{\text{ml of water}}{\text{sample weight}} \times 100$$

$$\% \text{ fat separation} = \frac{\text{ml of fat}}{\text{sample weight}} \times 100$$

Appendix Method 7

Peroxide Determination

1. A five-gram sample of fat is dissolved in a thirty milliliter mixture of acetic acid - chloroform solution (60% glacial acetic acid + 40 % chloroform).

2. To this solution is added 0.5 milliliter of saturated potassium iodine solution (10.4 g potassium iodide crystals in 5 ml of distilled hot water).

3. The solution is allowed to stand for exactly 1 minute and then 30 milliliters of distilled water is added.

4. This solution should be orange-yellow in color.

5. The solution is Titrate with 0.01 normal sodium thiosulfate until a light yellow color is obtained.

6. A few drops of a stable starch indicator is then added to give a bright blue color.

7. Titration is continued until the color disappears.

8. The formula for calculation :

$$\text{milli equivalents of peroxide per 1,000 g} = \frac{(\text{ml of Thiosulfate})\,(N)\,(1{,}000)}{\text{wt. in gram}}$$

Appendix Method 8

TBA (Thiobarbituric Acid) Test

1. Mix 10 g of blended meat with 49 ml of 50°C distilled water and 1 ml of sulfanilamide reagent (0.5% sulfanilamide in 20% HCl).
2. Quantitatively transfer into 800 ml Kjeldahl flask using 48 ml of 50°C distilled water. Add 2.0 ml HCl solution (1:2 solution of concentrated HCl and distilled water).
3. Add 5 drops of Dow antifoam A.
4. Turn on hot plate on Kjeldahl.
5. Turn on condenser water.
6. After the heating element is hot, place flask on the distillation unit and distill over 50 ml of solution (12 - 15 minutes).
7. Remove the distillate and replace with a beaker containing 400 ml of distilled water. Turn off heat and allow water to be drawn back through the apparatus. Turn off cooling water.
8. Add 5 ml of the distillate to a 50 ml Erlenmeyer flask (for the blank, use 5 ml distilled water).
9. Add 5 ml of TBA reagent (0.02 M-thiobarbituric acid in 90% glacial acetic acid) to both sample and blank.
10. Place string and cork in Erlenmeyer flask and place it in a boiling water bath for 35 minutes.
11. Cool in tap water for 10 minutes.
12. Place samples in Spectronic-20 tubes.
13. Measure OD (absorbance) on Spectronic-20 using a wavelength of 538 mu and the blank to obtain 100 % transmission.

Appendix Method 8 (Continued)

Preparation of Standard Curve

1. Dilute 0.22 g of tetraethyoxypropane (TEP) to 1,000 ml with distilled water. This will be a 10^{-3} M stock solution, which can be kept for 1 week in a refrigerator.

2. Each 1.0 ml of this stock solution contains 10^{-6} moles of TEP.

3. Pipet 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 ml of the stock solution into 50 ml volumetric flask and dilute each to 50 ml.

4. Transfer 5.0 ml of each of the distilled stock solution to a 50 ml Erlenmeyer flask.

5. Add 5.0 ml of TBA reagent to each flask.

6. Place a string and cork in each flask and place in boiling water bath for 35 minutes.

7. Cool in tap water for 10 minutes.

8. Transfer solutions to matched tubes and read absorbance of each tube at a wavelength of 538 mu. Use the solution containing no TEP to adjust the spectrophotometer to an absorbance of 0.0.

9. To obtain standard curve plot, absorbancy vs. moles of TEP.

Calculation of TBA Number

1. The TBA number is the mg of malonaldehyde per 1,000 g of sample and is calculated by multiplying the absorbance by a constant K. The K value is obtained from the standard curve and the known dilutions shown as follows :

$$K = \frac{S}{A} \times MW \times \frac{10}{W} \times \frac{100}{P}$$

where S = Standard concentration

Appendix Method 8 (Continued)

A = Absorbance of standard

MW = Molecular weight of malonaldehyde

P = Percent recovery

2. TBA number = K X Absorbance of sample.

Appendix Table 1 - Analyses of variance values for smokehouse yields and Instron shear values of bologna.

Source of Variation	df	Smokehouse Yield SS	MS	F	Instron Shear Value SS	MS	F
Rep (R)[a]	3	93.17	31.06	1.92$_{**}$	0.64	0.2	2.61$_{**}$
Salt (S)	1	576.00	576.00	35.57$_{*}$	7.39	7.39	90.76**
RxS Ea[b]	3	48.58	16.19	4.59$_{**}$	0.24	0.08	0.69$_{**}$
Casing (C)	1	95.06	95.06	32.39$_{**}$	37.88	37.88	42.23**
S x C	1	112.89	112.89	38.46$_{**}$	1.96	1.96	2.18
Temperature (T)	1	141.01	141.01	48.05$_{**}$	0.66	0.66	0.74
S x T	1	193.21	193.21	65.83$_{**}$	2.34	2.34	2.61$_{*}$
C x T	1	33.64	33.64	11.46$_{*}$	3.64	3.64	4.07$_{*}$
S x C x T	1	21.39	21.39	7.29*	4.82	4.82	5.37$_{*}$
R x C x T (S) Eb[c]	18	52.82	2.93	0.83	16.15	0.90	7.64*
Es[d]	32	112.99	3.53		3.76	0.12	
Total	63						

[a]Replications were smokehouse cycles in which a 0.75 and a 2.25% salt treatment were thermally processed.
[b]Error term used to test main effect of salt and replications.
[c]Experimental error term used to test split plot treatments.
[d]Sampling error term used to test experimental error.
** $p<0.01$.
* $p<0.05$.

Appendix Table 2 - Analyses of variance values for moisture and protein content of raw emulsions.

Source of Variation	df	Moisture Content SS	MS	F	Protein Content SS	MS	F
Diet (D)	1	14.30	14.30	97.25**	19.14	19.14	145.28**
Salt (S)	2	6.74	3.37	22.90**	10.64	5.32	40.38**
D x S	2	25.42	12.71	86.44**	27.92	13.96	105.97**
Temp. (T)	2	6.20	3.10	21.07**	0.31	0.16	1.19
D x T	2	0.51	0.26	1.73	0.13	0.07	0.52
S x T	4	1.13	0.28	1.93	12.74	3.18	24.18**
D x S x T	4	1.06	0.27	1.80	18.05	4.51	34.26**
Error	18	2.65	0.15		2.37	0.13	
Total	35						

** p<0.01.
* p<0.05.

Appendix Table 3 - Analyses of variance values for fat and ash content of raw emulsions.

Source of Variation	df	Fat Content SS	MS	F	Ash Content SS	MS	F
Diet (D)	1	0.34	0.34	1.36	0.12	0.12	13.37**
Salt (S)	2	4.13	2.07	8.20**	17.83	8.92	937.13**
D x S	2	27.18	13.59	54.01**	0.01	0.01	0.66
Temp. (T)	2	6.38	3.19	12.67**	0.01	0.01	0.21
D x T	2	0.89	0.45	1.77	0.03	0.02	1.35
S x T	4	9.90	2.45	9.84**	0.03	0.01	0.66
D x S x T	4	19.26	4.32	19.14**	0.05	0.01	1.25
Error	18	4.53	0.25		0.17	0.01	
Total	35						

** p<0.01.
* p<0.05.

Appendix Table 4 - Analyses of variance values for total water losses and oil losses of raw emulsions.

Source of Variation	df	Total Water Loss SS	MS	F	Total Oil Loss SS	MS	F
Diet (D)	1	240.25	240.25	326.38**	32.11	32.11	121.68**
Salt (S)	2	2604.50	1302.25	1769.09**	102.68	51.34	194.55**
D x S	2	307.17	153.59	208.64**	45.93	22.97	87.03**
Temp. (T)	2	47.79	23.90	32.46*	15.10	7.55	28.61
D x T	2	8.38	4.19	5.69*	0.68	0.34	1.29
S x T	4	11.58	2.90	3.93	0.15	0.04	0.14
D x S x T	4	6.58	1.37	2.24	1.40	0.35	1.33
Error	18	13.25	0.74		4.75	0.26	
Total	35						

** p<0.01.
* p<0.05.

Appendix Table 5 - Analyses of variance values for water binding capacity and emulsion stability of raw emulsions.

Source of Variation	df	Water Binding Capacity SS	MS	F	Emulsion Stability SS	MS	F
Diet(D)	1	516.73	516.73	1389.46*	448.03	448.03	379.51*
Salt(S)	2	2305.72	1152.86	3101.34*	3727.26	1863.63	1578.61*
D x S	2	246.03	123.02	330.78*	572.18	286.09	242.34*
Temp.(T)	2	127.96	63.98	172.04*	115.26	57.63	48.82
D x T	2	56.43	23.22	75.87*	4.76	2.38	2.02
S x T	4	49.25	12.31	33.11*	12.60	3.15	2.66
DxSxT	4	44.57	11.14	29.96	6.65	1.56	1.41
Error	18	6.69	0.37		21.25	1.18	
Total	35						

* p<0.05.

Appendix Table 6 - Analyses of variance values for moisture and protein content of bologna.

Source of Variation	df	Moisture Content SS	MS	F	Protein Content SS	MS	F
Diet (D)	1	128.58	128.58	191.74**	45.48	45.48	181.57**
Salt (S)	2	153.89	76.95	114.75**	51.54	25.77	102.88**
D x S	2	38.94	19.47	29.03**	24.87	12.44	49.64**
Casing (C)	1	66.59	66.59	99.31**	21.66	21.66	86.50*
D x C	1	1.28	1.28	1.91**	1.30	1.30	5.51**
S x C	2	27.09	13.55	20.20**	6.34	3.17	12.67*
D x S x C	2	11.74	5.87	8.76*	1.98	0.99	3.96**
Temp. (T)	1	4.96	4.96	7.41**	2.81	2.81	11.26**
D x T	1	0.12	0.12	0.19	0.10	0.10	0.41
S x T	2	0.04	0.02	0.03*	1.28	0.64	2.65*
D x S x T	2	7.52	3.76	5.61**	2.66	1.33	5.31**
C x T	1	0.27	0.27	0.40	0.35	0.35	1.41
D x C x T	1	0.05	0.05	0.09**	0.53	0.53	2.13**
S x C x T	2	12.45	6.25	9.28**	7.94	3.97	15.86**
DxSxCxT	2	11.54	5.77	8.61**	3.95	1.98	7.89**
Error	24	16.09	0.67		6.01	0.25	
Total	47						

** p<0.01.
* p<0.05.

Appendix Table 7 - Analyses of variance values for fat and ash content of bologna.

Source of Variation	df	Fat Content SS	MS	F	Ash Content SS	MS	F
Diet (D)	1	25.14	25.14	89.69**	0.08	0.08	2.02**
Salt (S)	2	138.41	69.21	246.90**	18.15	9.08	230.80**
D x S	2	31.12	15.56	55.52**	0.02	0.01	0.27
Casing (C)	1	10.70	10.70	38.20**	0.01	0.01	0.30
D x C	1	0.27	0.27	0.96	0.07	0.07	2.02
S x C	2	10.31	5.16	18.38**	0.13	0.07	1.63
D x S x C	2	6.10	3.05	10.90**	0.02	0.01	0.24
Temp. (T)	1	0.12	0.12	0.42	0.31	0.31	7.84**
D x T	1	0.81	0.81	2.89	0.02	0.02	0.48
S x T	2	3.45	1.78	6.16**	0.14	0.07	1.74
D x S x T	2	0.87	0.44	1.56	0.01	0.01	0.04
C x T	1	0.03	0.03	0.12	0.13	0.13	3.22
D x C x T	1	0.20	0.20	0.75	0.04	0.04	0.95
S x C x T	2	1.55	0.78	2.77	0.07	0.04	0.86
DxSxCxT	2	3.33	1.67	5.94**	0.03	0.02	0.36
Error	24	6.73	0.28		0.94	0.04	
Total	47						

** p<0.01.
* p<0.05.

Appendix Table 8 - Analyses of variance values for smokehouse yields and Instron shear values of bologna.

Source of Variation	df	Smokehouse Yield SS	MS	F	Instron Shear Value SS	MS	F
Diet (D)	1	704.86	704.86	359.67**	63.06	63.06	76.07**
Salt (S)	2	1857.18	928.59	473.82**	205.52	102.76	123.95**
D x S	2	151.36	75.63	38.62**	17.00	8.50	10.26**
Casing (C)	1	957.88	957.88	488.77**	7.05	7.05	8.50
D x C	1	190.42	190.42	97.16**	0.08	0.08	0.09
S x C	2	73.59	36.80	18.77**	0.66	0.33	0.40*
D x S x C	2	81.25	40.63	20.73**	5.78	2.89	3.49*
Temp. (T)	1	147.50	147.50	75.26**	5.71	5.71	6.89
D x T	1	33.74	33.74	17.21	2.21	2.21	2.66
S x T	2	6.50	3.25	1.66*	0.74	0.37	0.45
D x S x T	2	29.95	15.00	7.64	1.60	0.80	0.97
C x T	1	2.05	2.05	1.05*	1.73	1.73	2.09
D x C x T	1	9.25	9.25	4.72	0.44	0.44	0.53
S x C x T	2	4.20	2.10	1.07	0.01	0.01	0.01
DxSxCxT	2	12.10	6.05	3.09	0.55	0.28	0.33
Error	24	47.03	1.96		19.90	0.83	
Total	47						

** p<0.01.
* p<0.05.

Appendix Table 9 - Analyses of variance values for peroxide values and TBA numbers.

Source of Variation	df	Peroxide Value SS	MS	F	TBA Number SS	MS	F
Rep (R)	1	0.40	0.40	0.43	0.03	0.03	0.57
Fat (F)	2	3.58	1.79	1.89**	0.09	0.05	0.81
R x F Ea[a]	2	1.89	0.95	10.77	0.11	0.06	2.33*
Vacuum (V)	1	0.01	0.01	0.01**	0.18	0.18	5.19*
Time (T)	4	41.94	10.48	66.30	0.50	0.13	3.57*
F X V	2	1.05	0.53	3.34**	0.23	0.12	3.31**
F X T	8	4.76	0.60	3.76	1.95	0.25	6.87
V X T	4	0.31	0.08	0.51	0.47	0.12	3.28
F X V X T	8	1.94	0.24	1.54*	0.42	0.05	1.50
RxTxV(F) Eb[b]	26	4.11	0.16	2.62	0.92	0.04	1.48
Es[c]	59	5.47	0.09		1.42	0.02	
Total	117						

[a]Error term used to test main effect of salt and replications.
[b]Experimental error term used to test split plot treatments.
[c]Sampling error term used to test experimental error.
** p<0.01.
* p<0.05.

VITA

Yun-Chu Wu, son of Mr. Man-Chun Wu and Mrs. Shul-I Lee Wu, was born on November 16, 1954 in Taipei, Taiwan, the Republic of China. He attended Tunghai University in Taichung, Taiwan, where he received the Bachelor of Science Degree with honor in Animal Science in 1979. From 1979 to 1981, he attended the Ohio State University in Columbus, Ohio and received the Master of Science degree in Animal Science in 1981. In August of 1981, Wu was admitted into the Ph.D. program of the Department of Animal Science of Louisiana State University working with processed meat products. After being married to the former Ms. Peng-Ching Linda Chu on December 24, 1983, Wu and his wife had a son born on September 3, 1986. Wu will complete his Ph.D. degree in August, 1987.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Yun-Chu Wu

Major Field: Animal Science

Title of Dissertation: Influences of Different Processing Technologies on the Properties of Emulsion-Type Sausages

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

April 20, 1987